(54) Title: ADJUVANT COMBINATION FORMULATIONS

Cortical Abeta42 Levels
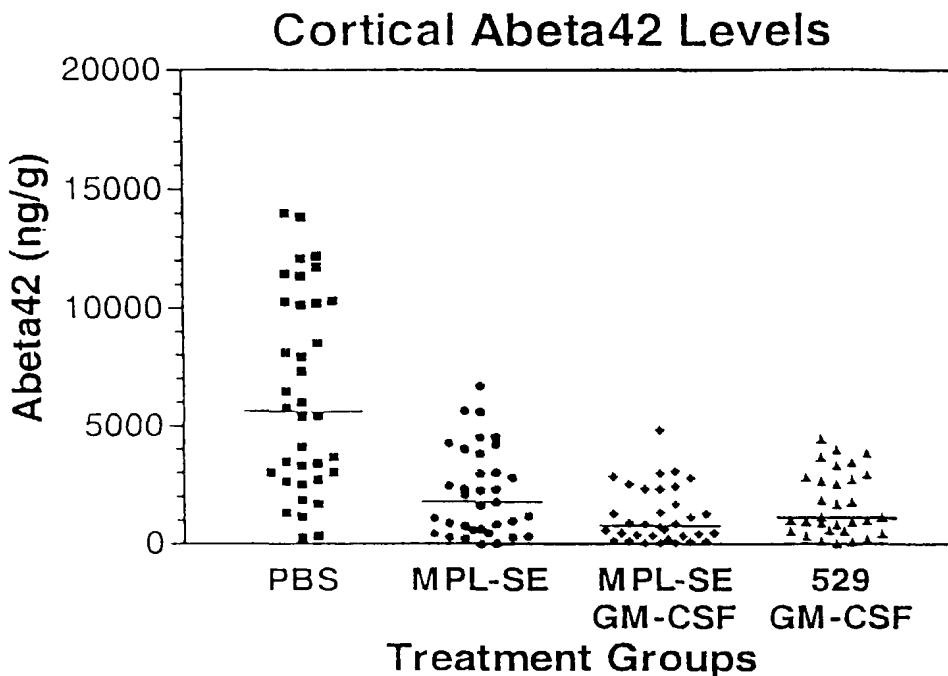
(57) Abstract: The use of an aminoalkyl glucosamine phosphate compound, or a derivative or analog thereof, in combination with a cytokine or lymphokine such as granulocyte macrophage colony stimulating factor or interleukin-12, is useful as an adjuvant combination in an antigenic composition to enhance the immune response in a vertebrate host to a selected antigen.

ADJUVANT COMBINATION FORMULATIONS

Field of the Invention

5   This invention relates to the use of an
aminoalkyl glucosamine phosphate compound, or a
derivative or analog thereof, in combination with a
cytokine or lymphokine, in particular granulocyte
macrophage colony stimulating factor or interleukin-12,
10   as an adjuvant formulation in an antigenic or
immunogenic composition to enhance the immune response
in a vertebrate host to a selected antigen.

Background of the Invention

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         The immune system uses a variety of
mechanisms for attacking pathogens. However, not all
of these mechanisms are necessarily activated after
immunization. Protective immunity induced by
20   immunization is dependent on the capacity of the
immunogenic composition to elicit the appropriate
immune response to resist or eliminate the pathogen.
Depending on the pathogen, this may require a cell-
mediated and/or humoral immune response.
25   The current paradigm for the role of helper T
cells in the immune response is that T cells can be
separated into subsets on the basis of the cytokines
they produce, and that the distinct cytokine profile
observed in these cells determines their function.
30   This T cell model includes two major subsets: TH-1
cells that produce interleukin-2 (IL-2) and interferon
gamma, which augment both cellular and humoral
(antibody) immune responses; and TH-2 cells that
produce interleukin-4, interleukin-5 and interleukin-10
35   (IL-4, IL-5 and IL-10, respectively), which augment
humoral immune responses (Bibliography entry 1).

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It is often desirable to enhance the immunogenic potency of an antigen in order to obtain a stronger immune response in the organism being immunized and to strengthen host resistance to the antigen-bearing agent. In some situations, it is desirable to shift the immune response from a predominantly humoral (TH-2) response to a more balanced cellular (TH-1) and humoral (TH-2) response.

A cellular response involves the generation of a CD8+ CTL (cytotoxic T-lymphocyte) response. Such a response is desirable for the development of immunogenic compositions against intracellular pathogens. Protection against a variety of pathogens requires strong mucosal responses, high serum titers, induction of CTL and vigorous cellular responses. These responses have not been provided by most antigen preparations, including conventional subunit immunogenic compositions. Among such pathogens is the human immunodeficiency virus (HIV).

Thus, there is a need to develop antigenic composition formulations that are able to generate both humoral and cellular immune responses in a vertebrate host.

Summary of the Invention

Accordingly, it is an object of this invention to utilize adjuvant combination formulations in antigenic compositions containing an aminoalkyl glucosamine phosphate compound (AGP), or a derivative or analog thereof, combined with a cytokine or lymphokine, in particular granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin-12 (IL-12), or an agonist or antagonist to said cytokine or lymphokine. In particular, the AGP is 2-[(R)-3-Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-

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phosphono-3-O-[(R)-3-tetradecanoyoxytetradecanoyl]-2-
[(R)-3-tetradecanoyoxytetradecanoylamino]-β-D-
glucopyranoside, which is also known as 529 (formerly
known as RC529).

5   An adjuvant is a substance that enhances the
immune response when administered together with an
immunogen or antigen. The adjuvant formulation of this
invention is administered together with a selected
antigen in an antigenic or immunogenic composition.
10   The antigenic compositions of this invention enhance
the immune response in a vertebrate host to that
selected antigen. The selected antigen may be a
polypeptide, peptide or fragment derived (1) from a
pathogenic virus, bacterium, fungus or parasite, or (2)
15   from a cancer cell or tumor cell, or (3) from an
allergen so as to interfere with the production of IgE
so as to moderate allergic responses to the allergen,
or (4) from amyloid precursor protein so as to prevent
or treat disease characterized by amyloid deposition in
20   a vertebrate host. In one embodiment of the invention,
the selected antigen is from HIV. The selected HIV
antigen may be an HIV protein, polypeptide, peptide or
fragment derived from said protein. In a particular
embodiment of the invention, the HIV antigen is a
25   specific peptide. In another embodiment of the
invention, the selected antigen is the β-amyloid
peptide (also referred to as Aβ peptide), which is an
internal, 39-43 amino acid fragment of amyloid
precursor protein (APP), which is generated by
30   processing of APP by the β and γ secretase enzymes.
        The AGP can be present as an aqueous
solution, or as a stabilized oil-in-water emulsion
(stable emulsion or SE). In a preferred embodiment of
the invention, the oil-in-water emulsion contains
35   squalene, glycerol and phosphatidyl choline. In the SE
formulation, the ACG is mixed with the cytokine or

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lymphokine to form the antigenic composition prior to
administration. The cytokine or lymphokine is not
required to enter the emulsion. In a preferred
embodiment of the invention, the AGP is in the SE form.
The antigenic composition may further comprise a
diluent or carrier.

        The invention is also directed to methods for
increasing the ability of an antigenic composition
containing a selected antigen (1) from a pathogenic
virus, bacterium, fungus or parasite to elicit the
immune response of a vertebrate host, or (2) from a
cancer antigen or tumor-associated antigen from a
cancer cell or tumor cell to elicit a therapeutic or
prophylactic anti-cancer effect in a vertebrate host,
or (3) from an allergen so as to interfere with the
production of IgE so as to moderate allergic responses
to the allergen, or (4) from a molecule or portion
thereof which represents those produced by a host (a
self molecule) in an undesired manner, amount or
location so as to reduce such an undesired effect, by
including an effective adjuvanting amount of a
combination of a cytokine or lymphokine, in particular
an AGP with GM-CSF or IL-12, or an agonist or
antagonist to said cytokine or lymphokine.

        The invention is further directed to methods
for increasing the ability of an antigenic composition
containing a selected antigen from a pathogenic virus,
bacterium, fungus or parasite to elicit cytotoxic T
lymphocytes in a vertebrate host by including an
effective adjuvanting amount of a combination of a
cytokine or lymphokine, in particular an AGP with GM-
CSF or IL-12, or an agonist or antagonist to said
cytokine or lymphokine.

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Brief Description of the Figures

Figure 1 depicts the geometric mean titers of antibodies to the Aβ1-42 peptide in transgenic mice immunized as follows: Group 1 - Aβ1-42 peptide plus PBS (not shown); Group 2 - Aβ1-42 peptide plus MPL™ SE (squares); Group 3 - Aβ1-42 peptide plus MPL™ SE and GM-CSF (triangles); Group 4 - Aβ1-42 peptide plus 529 SE and GM-CSF (inverted triangles).

Figure 2 depicts total Aβ cortical levels in transgenic mice immunized with the four Groups described for Figure 1.

Figure 3 depicts Aβ1-42 peptide cortical levels in transgenic mice immunized with the four Groups described for Figure 1.

Figure 4 depicts the frontal cortex amyloid burden in transgenic mice immunized with the four Groups described for Figure 1.

Figure 5 depicts the frontal cortex neuritic burden in transgenic mice immunized with the four Groups described for Figure 1.

Figure 6 depicts the retrosplenial cortex astrocytosis levels in transgenic mice immunized with the four Groups described for Figure 1.

Figure 7 depicts the HIV C4(E9V)-V3$_{89.6P}$ peptide-specific IgG geometric mean antibody titers in serum in two groups of cynomologous macaques (four animals per group). Group 1 animals were immunized intranasally with the C4(E9V)-V3$_{89.6P}$ peptide alone. Group 2 animals were immunized intramuscularly with the C4(E9V)-V3$_{89.6P}$ peptide formulated with 529 SE and GM-CSF. Arrows indicate the immunizations at weeks 0, 4, 8, 18 and 23.

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Figure 8 depicts the geometric mean antibody titers in cervicovaginal lavage samples of the same animals described for Figure 7.

Figure 9 depicts the geometric mean antibody titers in nasal wash samples of the same animals described for Figure 7.

Detailed Description of the Invention

Adjuvants, cytokines and lymphokines are immune modulating compounds which have the ability to enhance and steer the development and profile of immune responses against various antigens that are themselves poorly immunogenic. The appropriate selection of adjuvants, cytokines and lymphokines can induce good humoral and cellular immune responses that would not develop in the absence of adjuvant, cytokine or lymphokine. In particular, adjuvants, cytokines and lymphokines have significant effects in enhancing the immune response to subunit and peptide antigens in immunogenic compositions. Their stimulatory activity is also beneficial to the development of antigen-specific immune responses directed against protein antigens. For a variety of antigens that require strong mucosal responses, high serum titers, induction of CTL and vigorous cellular responses, adjuvant and cytokine/lymphokine combinations provide stimuli that are not provided by most antigen preparations.

Numerous studies have evaluated different adjuvant formulations in animal models, but alum (aluminum hydroxide or aluminum phosphate) is currently the only adjuvant licensed for widespread use in humans. Another adjuvant is Stimulon™ QS-21 (QS-21) (Antigenics Inc., Framingham, MA (2)). One group of adjuvants, stable emulsions, consisting of various water-in-oil or oil-in-water combinations, has received

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considerable attention for their immunopotentiating
ability. These formulations generally consist of
various combinations of metabolizable or inert oils,
that act to stabilize and depot antigen at the site of
5   injection. One such adjuvant is incomplete Freund's
adjuvant (IFA), which includes mineral oil, water and
an emulsifying agent. Complete Freund's adjuvant (CFA)
is IFA plus heat-killed Mycobacteria. A particular
concern in using these types of adjuvants has been
10   injection site-associated irritation, often the result
of mononuclear cell infiltrations causing granulomatous
lesions. Therefore, other compounds and formulations
are being investigated as potential adjuvants.

One group of such compounds are the
15   aminoalkyl glucosamine phosphate compounds (AGPs),
which are described in United States Patent Number
6,113,918, for example at column 2, line 14-column 3,
line 38, which is hereby incorporated by reference (3).
AGPs have an aminoalkyl (aglycon) group which is
20   glycosidically linked to a 2-deoxy-2-amino-α-D-
glucopyranose (glucosamine) to form the basic
structure. Further substituents include the
phosphorylation of the 4 or 6 carbon on the glucosamine
ring and three 3-alkanoyloxyalkanoyl residues.

25   One such AGP is the compound designated 529
(whose full chemical name is 2-[(R)-3-
Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-
phosphono-3-O-[(R)-3-tetradecanoyoxytetradecanoyl]-2-
[(R)-3-tetradecanoyoxytetradecanoylamino]-β-D-
30   glucopyranoside), which is produced by Corixa
(Hamilton, MT).

Corixa also has formulated a metabolizable
oil-in-water formulation which, when combined with 529,
results in the formation of a stabilized emulsion
35   designated 529 SE. The stabilized emulsion is
generated through microfluidization of 529 with

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squalene oil, glycerol and phosphatidyl choline. The
current formulation is a GMP-quality microfluidized
emulsion. Emulsions containing 1% oil (although other
concentrations may be used) are described in the
5   experiments below.

       529 SE resulted in no discernable gross
tissue pathology when administered subcutaneously into
Balb/c or Swiss-Webster mice. A stabilized emulsion
containing the same components, but without 529 was
10   also generated for comparative purposes. Specifically,
subcutaneous immunization with a cysteine-deleted 39
amino acid version (-Cys) of a 40 amino acid HIV
peptide T1SP10MN(A) (which lacks the cysteine residue
at amino acid number 17 of the 40 amino acid peptide
15   (+Cys)), or with Aβ1-42 (an internal, 42 amino acid
fragment of APP), each formulated with the combination
of adjuvants 529 SE and GM-CSF, resulted in no
discernable inflammation, redness, swelling or
induration.

20   Also within the scope of this invention are
derivatives and analogs of 529 and other AGPs. Such
compounds include, but are not limited to the compounds
described in United States Patent Number 6,113,918 (3).

       The incorporation of cytokines and
25   lymphokines into immunogenic compositions has shown
promise for the expansion and enhancement of the
potential of immunogenic compositions (4). The
cytokine interleukin-12 (IL-12) has been demonstrated
to evoke and enhance cell mediated immunity, through a
30   shift in T helper cell subset expansion towards a Th1
cytokine profile (i.e., to IgG2 subclass in the mouse
model) (5-7). In mice, recombinant murine IL-12 has
been shown to enhance a Th1 dominated immune response
profile (4).

35   IL-12 is produced by a variety of antigen-
presenting cells, principally macrophages and

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monocytes. It is a critical element in the induction
of TH1 cells from naïve T-cells. Production of IL-12
or the ability to respond to it has been shown to be
critical in the development of protective TH1-like
5   responses, for example, during parasitic infections,
most notably Leishmaniasis (8), as well as enhancing
the cell mediated immune response to an antigen from a
pathogenic bacterium or virus (9) or from a cancer cell
(10). The effects of IL-12 are mediated in large part
10   by interferon-gamma produced by NK cells and T helper
cells. Interferon-gamma is critical for the induction
of IgG2a antibodies to T-dependent protein antigens
(11) and IgG3 responses to T-independent antigens (12).
IL-12, originally called natural killer cell
15   stimulatory factor, is a heterodimeric cytokine (13).
The expression and isolation of IL-12 protein in
recombinant host cells is described in published
International Patent Application WO 90/05147 (14).
        Another cytokine that holds potential promise
20   as an adjuvant is GM-CSF. GM-CSF is a particular type
of colony stimulating factor (CSF). The CSFs are a
family of lymphokines that induce progenitor cells
found in the bone marrow to differentiate into specific
types of mature blood cells. As described in U.S.
25   Patent Number 5,078,996 (15), which is hereby
incorporated by reference, GM-CSF activates macrophages
or precursor monoctyes to mediate non-specific
tumoricidal activity. The nucleotide sequence encoding
the human GM-CSF gene has been described (15). A
30   plasmid containing GM-CSF cDNA has been transformed
into E. coli and has been deposited with the American
Type Culture Collection (ATCC), 10801 University
Boulevard, Manassas, VA 20110-2209, under Accession
Number 39900. As described in U.S. Patent Number
35   5,229,496 (16), which is hereby incorporated by
reference, the GM-CSF gene has also been inserted into

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a yeast expression plasmid and deposited with the ATCC
under Accession Number 53157. Furthermore, as
described in U.S. Patent Number 5,073,627 (17), which
is hereby incorporated by reference, a DNA sequence
encoding GM-CSF having glycosylation sites removed was
deposited with the ATCC under Accession Number 67231.

GM-CSF has been shown to upregulate protein
molecules on antigen presenting cells known to enhance
immune responses (18), and to affect Ig secretion in
sort-purified murine B cells (19). GM-CSF has also
been described as an adjuvant for immunogenic
compositions (20).

Other cytokines or lymphokines have been
shown to have immune modulating activity, including,
but not limited to, the interleukins 1-alpha, 1-beta,
2, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16, 17 and 18, the
interferons-alpha, beta and gamma, granulocyte colony
stimulating factor, and the tumor necrosis factors
alpha and beta.

Of concern related to the systemic
administration of any cytokine or lymphokine are the
biological consequences associated with cytokine or
lymphokine activity. Additionally, cytokine or
lymphokine effects related to the development of
antigen-specific immune responses should be enhanced if
local concentrations of cytokine or lymphokine are
maintained.

The combinations of 3-O-deacylated
monophosphoryl lipid A or monophosphoryl lipid A with
GM-CSF or IL-12 have been evaluated; enhancement of
various immune response parameters was observed (21).

The invention described herein demonstrates
that, through the combination of an antigen, selected
cytokine or lymphokine adjuvant, and the second
adjuvant, an AGP (preferably in a stable metabolizable

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emulsion), the immune responses specific for the antigen are enhanced.

The antigens selected for inclusion in the antigenic compositions of this invention comprise peptides or polypeptides derived from proteins, proteins, as well as fragments of any of the following: saccharides, proteins, poly- or oligonucleotides, or other macromolecular components. As used herein, a "peptide" comprises a series of at least three amino acids and contains at least one antigenic determinant or epitope, while a "polypeptide" is a longer molecule than a peptide, but does not constitute a full-length protein. Such peptides, polypeptides or proteins may be conjugated to an unrelated protein, such as tetanus toxoid or diphtheria toxoid. As used herein, a "fragment" comprises a portion, but less than all of a saccharide, protein, poly- or oligonucleotide, or other macromolecular components. In the case of HIV, the antigenic compositions of this invention further comprise full-length HIV proteins.

The invention is first exemplified in a model system using peptide antigens derived from HIV. These peptides are described in or derived from U.S. Patent Numbers 5,013,548 (22) and 5,019,387 (23), which are hereby incorporated by reference and are now summarized. These peptides comprise amino acid sequences which correspond to a region of the HIV envelope protein against which neutralizing antibodies and T cell responses are produced.

HIV is a human retrovirus which is the causative agent of acquired immunodeficiency syndrome (AIDS). HIV infects T lymphocytes of the immune system by attaching its external envelope glycoprotein to the CD4 (T4) molecule on the surface of T lymphocytes, thus using the CD4 (T4) molecule as a receptor to enter and infect T cells. Attempts to induce a protective immune

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response specific for HIV-infection through
immunization have been met with very limited success.
A number of approaches are currently being pursued in
an attempt to determine an effective and protective
strategy for the development of immunogenic
compositions. These include using attenuated and
recombinant bacterial vectors that express antigenic
epitopes from HIV (24), recombinant adenovirus (25) or
vaccinia virus vectors (26), DNA immunization (27), and
synthetic peptides that contain various T and B cell
epitopes of HIV (28).

        The HIV external envelope glycoprotein gp120
has been shown to be capable of inducing neutralizing
antibodies in man. The recombinant protein PB1, which
encodes approximately one-third of the entire gp120
molecule, has been shown to include the part of the
envelope protein that induces the formation of
neutralizing antibodies. However, studies in
chimpanzees demonstrated that neither intact gp120 or
PB1 is able to induce the production of high titers of
neutralizing antibodies.

        Short peptides were synthesized by
conventional methods which correspond to antigenic
determinants of gp120 and generate an antibody response
against gp120 that neutralize the virus and induce T-
helper and CTL responses against the virus.

        One such peptide is the C4/V3 multiepitope-
containing HIV-1$_{MN}$ peptide designated
T1SP10MN(A)(+Cys), and a cysteine-deleted variant
T1SP10MN(A)(-Cys). These peptides include Th, T_{CTL} and
B epitopes, but do not induce antibodies which
interfere with CD4 binding. Previously, it has been
demonstrated that these C4/V3 HIV peptides are
promising candidates for the induction of immune
responses when administered with CFA, or CFA-like
adjuvants (29-34). These peptides contain epitopes

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that have previously been shown to evoke CD4+ Th cell
responses in both mice and humans, and it contains both
a principal neutralizing determinant and a site which
is recognized by CD8+ CTL in both Balb/c mice and
5   humans that are HLA B7+. The 39 amino acid peptide has
recently demonstrated both immunogenicity and safety in
HIV-infected patients (28).
        T1SP10MN(A)(+Cys) has the following sequence
of 40 amino acids:
10   Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile
His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (31)
(SEQ ID NO:1).
        T1SP10MN(A)(-Cys) has been synthesized
15   without the cysteine at position 17 and has the
following sequence of 39 amino acids:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
20   NO:2).
        This cysteine residue is located outside of
the functional epitopes recognized by Th cells, CTL or
B cells. Other HIV peptides from various regions of
the viral genome are described in U.S. Patent Number
25   5,861,243 (35), U.S. Patent Number 5,932,218 (36), U.S.
Patent Number 5,939,074 (37), U.S. Patent Number
5,993,819 (38), U.S. Patent Number 6,037,135 (39),
Published European Patent Application Number 671,947
(40), and U.S. Patent Number 6,024,965 (41), which are
30   also incorporated by reference.
        A 28 amino acid peptide conjugate designated
ST1/p11C is also used. The conjugate consists of a 16
amino acid SIV env-derived T-helper peptide designated
ST-1, conjugated to a 12 amino acid SIV mac 251 Gag
35   peptide (amino acids 179-190 of Gag) designated p11C
(42). The p11C peptide in tetrameric form has

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demonstrated CTL activity in SIV mac-infected Mamu-A*01
rhesus monkeys (43). The ST1-p11C peptide conjugate
has the following amino acid sequence:

          Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly
          Lys Asn Val Tyr Leu Glu Gly Cys Thr Pro Tyr
          Asp Ile Asn Gln Met Leu (SEQ ID NO:3);

A 39 amino acid peptide conjugate designated C4-V3$_{89.6P}$
(44) is also used. The C4 region of this peptide
conjugate (16 amino acids) is derived from the fourth
constant region of the HIV-1 envelope protein and
represents a universal T-helper epitope. The V3
portion of the peptide (23 amino acids) is derived from
the third hypervariable region of the HIV-1 envelope
protein and represents a critical neutralizing
determinant. The C4-V3$_{89.6P}$ conjugate has the following
amino acid sequence:

          Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly
          Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn
          Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg
          Ala Phe Tyr Ala Arg Arg (SEQ ID NO:4).

          The HIV antigen may be a protein,
polypeptide, peptide or fragment derived from said
protein. The protein may be a glycoprotein such as
gp41, gp120 or gp160. Alternatively, the protein may
be a protein encoded by such genes as *gag*, *pol*, *vif*,
rev, *vpr*, *tat*, *nef* or *env*. Peptides derived from such
proteins will contain at least one antigenic
determinant (epitope) at least six amino acids in
length.
          The immune response to an HIV peptide may be
enhanced by covalently linking (conjugating) the
peptide to a pharmaceutically acceptable carrier
molecule. Examples of suitable carrier molecules
include tetanus toxoid, diphtheria toxoid, keyhole

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limpet haemocyanin and other peptides corresponding to
T cell epitopes of the HIV gp120 glycoprotein.

It is currently felt that a successful
strategy for immunization against HIV will need to
elicit mucosal immunity to HIV, as well as a good CTL
response. In a recent murine study using the
T1SP10MN(A) multi-epitope peptide, and a mucosal
adjuvant, cholera toxin, it was shown that intranasal
immunization induced neutralizing serum IgG1 antibodies
(45). A subsequent study also using HIV-V3 loop
peptides demonstrated the induction of mucosally
synthesized IgA antibody and strong cell mediated
responses, including peptide-specific CTL (46). The
functional role of high titers of systemic and
neutralizing antibodies in the prevention of, or
stabilization of HIV-infected individuals is unknown,
although high titers of virus-specific antibody are
believed to be important in preventing viral spread.

In a preferred embodiment of the invention, a
stable oil-in-water emulsion is formulated which
contains 529, which is then mixed with the cytokines
IL-12 or GM-CSF. The data presented below demonstrate
that the combination of 529 plus GM-CSF results in high
titers of HIV-neutralizing serum antibodies. The
combination of 529 SE and GM-CSF induces high titers of
antigen-specific IgG antibodies, including both IgG1
and IgG2a subclasses, in the vaginal vault of immunized
female mice. Immunization of mice with the T1SP10MN(A)
(-Cys) peptide formulated with 529 SE and GM-CSF
induced a strong cellular immune response as determined
by enhanced antigen specific cellular proliferation and
secretion into culture of cytokines, as well as the
induction of peptide-specific CTL responses. Similar
results were seen when mice were immunized with the
Aβ1-42 peptide from APP with 529 SE and GM-CSF.

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Generally, the antigen/adjuvant formulation of 529 or 529 SE combined with GM-CSF or IL-12 and a protein or peptide of choice induces high titers of antigen-specific and virus neutralizing antibody, a significant shift in the IgG subclass ratio to a greater proportion of complement-fixing IgG antibodies (in favor of IgG2a in mice), elevated production of cytokines and cellular proliferation from mononuclear cells in response to antigen stimulation *in vitro*. These properties were not observed with formulations of antigen and SE in the absence of 529, either with or without GM-CSF or IL-12. The formulations of this invention also induce good cellular responses as determined through induction of CTL.

A benefit of 529 SE is that the formulation does not induce granulomatous accumulation and inflammation at the site of injection; such injection site reactions are typically induced by water-in-oil or oil-in-water adjuvant formulations.

An experiment was conducted to compare the administration of the HIV peptide T1SP10MN(A)(-Cys) with 529 SE alone, or with 529 SE plus IL-12, or 529 SE plus GM-CSF.

In this experiment (Table 1 below), Balb/c mice immunized subcutaneously with the HIV peptide T1SP10MN(A)(-Cys), formulated with 529 SE, elicited peptide-specific serum IgG titers after only two injections. The IgG1 and IgG2a subclass titers were also elicited. The inclusion of a second adjuvant, either GM-CSF or IL-12, boosted the IgG total titers, as well as the IgG1 subclass titers. The addition of IL-12 boosted the IgG2a subclass titer; the addition of GM-CSF did not boost the IgG2a subclass titer.

In another experiment, as a measure of functional cell mediated immunity, the ability of spleen cells from mice immunized with 529 SE, or 529 SE

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plus IL-12, or 529 SE plus GM-CSF, formulated together
with the multi-epitope peptide T1SP10MN(A)(-Cys) to
generate HIV_{MN}-specific CTL responses was assessed.

As shown in Table 2, spleen cells from mice
immunized with any of the adjuvants demonstrated low
activity toward target cells that were either
unlabelled or pulse-labeled with the irrelevant IIIB
CTL epitope. HIV_{MN}-specific CTL-mediated target cell
lysis was markedly enhanced when 529 SE plus IL-12 was
administered compared to 529 SE alone, and still
further enhanced when 529 SE plus GM-CSF was
administered (Table 2).

In still another experiment, rhesus monkeys
were immunized with the ST1-p11C or $C4-V3_{89.6P}$ peptides
and IFA or 529 SE plus GM-CSF (groups shown in Table
15). The results of the analyses are shown in Table
16-22 and are now summarized.

The ST1-p11C peptide formulation itself
seemed to be well tolerated in all the animals tested.
However, significant injection site reactivities were
noted with the adjuvant IFA. In addition, possible
minor adverse effects of the adjuvant formulation 529
SE/GM-CSF were seen immediately after the final
immunization. The ST1-p11C peptide formulation
containing IFA was capable of inducing a potent p11C-
specific cellular immune response in one of two Mamu-
A*01 positive rhesus monkeys tested. The ST1-p11C
peptide formulation containing 529 SE/GM-CSF was also
capable of inducing a p11C-specific cellular immune
response in one of two Mamu-A*01 positive rhesus
monkeys tested.

The $C4-V3_{89.6P}$ peptide formulation containing
IFA was capable of generating peak plasma ELISA
antibody titers in the range of 1:25,600 - 1:102,400
and serum neutralizing antibody titers against $SHIV_{89.6}$
and $SHIV_{89.6P}$. The $C4-V3_{89.6P}$ peptide formulation

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containing 529 SE/GM-CSF was capable of generating peak
plasma ELISA antibody titers in the range of 1:6,400 -
1:12,800 and low level neutralizing antibody responses
to SHIV$_{89.6}$, but not to SHIV$_{89.6P}$.

5   Given the small number of animals per group
(two), it is difficult to draw concrete conclusions.
However, the level of the immune response, both humoral
and cellular, generated by both peptide formulations
containing 529 SE/GM-CSF was qualitatively lower than

10   the immune response seen in the animals adjuvanted with
IFA. It must always be noted that IFA is not an
approved component in compositions for commercial use
in humans. In addition, there is some limited evidence
that the functional properties and the phenotype (i.e.

15   cytokine profiles) of the responding cells might be
different depending on the adjuvant formulation used.

       In yet another experiment, animals from a
second primate species, cynomologous macaques, were
immunized with the C4-V3$_{89.6P}$ peptide that had been

20   modified by changing the glutamic acid at amino acid
residue 9 to valine. The resulting peptide conjugate,
designated C4(E9V)-V3$_{89.6P}$, has the following sequence:

       Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly
       Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn

25   Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg
       Ala Phe Tyr Ala Arg Arg (SEQ ID NO:5).

Animals were immunized with the C4(E9V)-V3$_{89.6P}$ peptide,
either without adjuvant or with the combination of 529
SE plus GM-CSF.

30   The results indicate that the C4(E9V)-V3$_{89.6P}$
peptide when administered by intramuscular injection in
combination with 529 SE/GM-CSF elicits significantly
higher peptide-specific IgG titers in serum than the
same amount of the C4(E9V)-V3$_{89.6P}$ peptide administered

35   intranasally without adjuvant (Figures 7-9). The
results from this experiment clearly demonstrate that

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an HIV peptide immunogen when administered in
combination with the appropriate combination of
adjuvants is capable of eliciting systemic humoral
immunity.

5   Desirable immunogenic compositions for
preventing or treating disease characterized by amyloid
deposition (a self molecule) in a vertebrate host,
which contain the adjuvant combinations of this
invention, include those containing portions of the

10   beta amyloid precursor protein (APP). This disease is
referred to variously as Alzheimer's disease,
amyloidosis or amyloidogenic disease. The β-amyloid
peptide (also referred to as Aβ peptide) is an
internal, 39-43 amino acid fragment of APP, which is

15   generated by processing of APP by the β and γ secretase
enzymes. The Aβ1-42 peptide has the following
sequence:
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His
Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys

20   Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala
(SEQ ID NO:6).

          In some patients, the amyloid deposit takes
the form of an aggregated Aβ peptide. Surprisingly, it
has now been found that administration of isolated Aβ

25   peptide induces an immune response against the Aβ
peptide component of an amyloid deposit in a vertebrate
host (47). Thus, the immunogenic compositions of this
invention include the adjuvant combinations of this
invention plus Aβ peptide, as well as fragments,

30   derivatives or modifications of Aβ peptide and
antibodies to Aβ peptide or fragments, derivatives or
modifications thereof. One such fragment of Aβ peptide
is the 28 amino acid peptide having the following
sequence (48):

35   Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His

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Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
(SEQ ID NO:7).

Other fragments of the Aβ peptide which are
of interest include, but are not limited to, amino
acids 1-10, 1-7, 1-6, 1-5, 3-7, 1-3 and 1-4, which may
be administered in an unconjugated form, or conjugated
to an unrelated protein.

A series of studies was conducted with the
Aβ1-42 peptide and various adjuvants. A summary of the
results will now be presented.

In a first experiment, Swiss-Webster mice
immunized subcutaneously in the rump with the Aβ1-42
peptide generated peptide-specific antibody IgG, IgG1
and IgG2a titers, demonstrating that the Aβ1-42 peptide
is a viable candidate antigen. Addition of GM-CSF to
529 SE and the Aβ1-42 peptide resulted in elevated
serum antibody IgG, IgG1 and IgG2a titers compared to
recipients of 529 SE and the Aβ1-42 peptide (see Tables
3-8). The serum antibodies from individual mice
receiving the combination of 529 SE plus GM-CSF were
elevated in more instances and were elevated more
quickly than individual mice receiving 529 SE alone
(data not shown). When this first experiment was
repeated with older Swiss-Webster mice (6-8 months
instead of less than 3 months), similar results to
those in Tables 3-8 were seen (data not shown).

In a second experiment, Swiss-Webster mice
were immunized subcutaneously in the rump with the Aβ1-
42 peptide and 529 SE, with varying amounts of GM-CSF.
IgG endpoint titers increased in a dose dependent
manner as the amount of GM-CSF increased (from 0.1 to 1
to 10μg) (Table 9). The IgG titers for all
combinations of 529 SE plus GM-CSF were higher than for
groups receiving another adjuvant, QS-21, alone or with
GM-CSF. IgG1 subclass titers were also increased for

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the various 529 SE plus GM-CSF groups compared to a
group which received 529 SE plus GM-CSF in a first dose
and 529 SE alone in a second dose (Table 10). IgG2a
subclass titers were also increased for the various 529
SE plus GM-CSF groups compared to the 529 SE alone
group in a dose dependent manner (Table 11).

In a third experiment, Swiss-Webster mice
were immunized subcutaneously in the rump with the Aβ1-
42 peptide and 529 SE, with or without varying amounts
of GM-CSF. IgG endpoint titers were increased for the
various 529 SE plus GM-CSF groups (0.5 to 2 to 5 to
10μg), although not in a dose dependent manner (Table
12). Both IgG1 and IgG2a subclass titers were also
increased for the various 529 SE plus GM-CSF groups
compared to the 529 SE alone group, although not in a
dose dependent manner (Tables 13 [IgG1] and 14
[IgG2a]).

In a fourth experiment, transgenic mice were
used which express a variant form of the β-amyloid
precursor protein (APP) having a mutation at residue
717, with valine substituted by phenylalanine (49).
This mutation is associated with familial Alzheimer's
disease in humans. These transgenic mice (designated
PDAPP mice) progressively develop many of the
pathological hallmarks of Alzheimer's disease,
including Aβ deposits, neuritic plaques and
astrocytosis, and thus serve as an animal model for
human Alzheimer's disease.

In this fourth experiment, PDAPP mice were
immunized subcutaneously with the Aβ1-42 peptide with
or without various adjuvants and at the dosages shown
in Table A. Specifically, Group 1 mice received the
Aβ1-42 peptide with MPL™ (Corixa, Hamilton, MT) in
stable emulsion form (SE) as a positive control; Group
2 mice received the Aβ1-42 peptide with MPL™ SE plus

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murine GM-CSF; Group 3 mice received the Aβ1-42 peptide
with 529 SE plus murine GM-CSF; Group 4 mice received
PBS as a negative control. Groups 2 and 3 exhibited a
more rapid increase in anti-Aβ1-42 antibody titer
values as well as a higher peak titer than Groups 1 or
4. However, the titers in Groups 2 and 3 fell back to
the equivalent titer of Group 1 positive controls
within 2-3 months (Figure A). Groups 1, 2 and 3 showed
significant lowering of brain Aβ levels as measured by
ELISA (Tables B-C and Figures B-C), lower amyloid
burden (Table D and Figure D) and less neuritic
dystrophy (Table E and Figure E), when compared to the
Group 4 negative controls. Groups 2 and 3 had a
significant reduction in astrocytosis compared to the
Group 1 positive controls (Figure F).

Thus, the adjuvanting properties of 529 SE
and GM-CSF or IL-12 appear to be synergistic when
formulated together.

The antigenic compositions of the present
invention modulate the immune response by improving the
vertebrate host's antibody response and cell-mediated
immunity after administration of an antigenic
composition comprising a selected antigen from a
pathogenic virus, bacterium fungus or parasite, and an
effective adjuvanting amount of AGP such as 529 (in an
aqueous or stable emulsion form) combined with a
cytokine or lymphokine, in particular GM-CSF or IL-12.
Other cytokines or lymphokines have been shown to have
immune modulating activity, including, but not limited
to, the interleukins 1-alpha, 1-beta, 2, 4, 5, 6, 7, 8,
10, 13, 14, 15, 16, 17 and 18, the interferons-alpha,
beta and gamma, granulocyte colony stimulating factor,
and the tumor necrosis factors alpha and beta.

Agonists of or antagonists to certain
cytokines or lymphokines are also within the scope of
this invention. As used herein, the term "agonist"

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means a molecule that enhances the activity of, or
functions in the same way as, said cytokines or
lymphokines. An example of such an agonist is a mimic
of said cytokines or lymphokines. As used herein, the
term "antagonist" means a molecule that inhibits or
prevents the activity of said cytokines or lymphokines.
Examples of such antagonists are the soluble IL-4
receptor and the soluble TNF receptor.

 As used herein, the term "effective
adjuvanting amount" means a dose of the combination of
adjuvants described herein, which is suitable to elicit
an increased immune response to a selected antigen in a
vertebrate host, compared to a host receiving that
selected antigen in the absence of the adjuvant
combination. The particular dosage will depend in part
upon the age, weight and medical condition of the host,
as well as on the method of administration and the
antigen. In a preferred embodiment, the combination of
adjuvants will utilize 529 in the range of 0.1-
500μg/dose; in a more preferred embodiment, the range
is 1-100μg/dose. Suitable doses are readily determined
by persons skilled in the art. The antigenic
compositions of this invention may also be mixed with
immunologically acceptable diluents or carriers in a
conventional manner to prepare injectable liquid
solutions or suspensions.

 The antigenic or immunogenic compositions of
this invention are administered to a human or non-human
vertebrate by a variety of routes, including, but not
limited to, intranasal, oral, vaginal, rectal,
parenteral, intradermal, transdermal (see, e.g.,
International application WO 98/20734 (50) which is
hereby incorporated by reference), intramuscular,
intraperitoneal, subcutaneous, intravenous and
intraarterial. The amount of the antigen component or
components of the antigenic composition will vary

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depending in part upon the identity of the antigen, as
well as upon the age, weight and medical condition of
the host, as well as on the method of administration.
Again, suitable doses are readily determined by persons
skilled in the art. It is preferable, although not
required, that the antigen and the combination of
adjuvants be administered at the same time. The number
of doses and the dosage regimen for the antigenic
composition are also readily determined by persons
skilled in the art. In some instances, the adjuvant
properties of the combination of adjuvants may reduce
the number of doses needed or the time course of the
dosage regimen.

The combinations of adjuvants of this
invention are suitable for use in antigenic or
immunogenic compositions containing a wide variety of
antigens from a wide variety of pathogenic
microorganisms, including but not limited to those from
viruses, bacteria, fungi or parasitic microorganisms
which infect humans and non-human vertebrates, or from
a cancer cell or tumor cell. The antigen may comprise
peptides or polypeptides derived from proteins, as well
as fragments of any of the following: saccharides,
proteins, poly- or oligonucleotides, cancer or tumor
cells, allergens, self molecules (such as amyloid
precursor protein), or other macromolecular components.
In some instances, more than one antigen is included in
the antigenic composition.

Desirable viral immunogenic compositions
containing the adjuvant combinations of this invention
include those directed to the prevention and/or
treatment of disease caused by, without limitation,
Human immunodeficiency virus, Simian immunodeficiency
virus, Respiratory syncytial virus, Parainfluenza virus
types 1-3, Influenza virus, Herpes simplex virus, Human
cytomegalovirus, Hepatitis A virus, Hepatitis B virus,

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Hepatitis C virus, Human papillomavirus, poliovirus, rotavirus, caliciviruses, Measles virus, Mumps virus, Rubella virus, adenovirus, rabies virus, canine distemper virus, rinderpest virus, Human metapneumovirus, avian pneumovirus (formerly turkey rhinotracheitis virus), Hendra virus, Nipah virus, coronavirus, parvovirus, infectious rhinotracheitis viruses, feline leukemia virus, feline infectious peritonitis virus, avian infectious bursal disease virus, Newcastle disease virus, Marek's disease virus, porcine respiratory and reproductive syndrome virus, equine arteritis virus and various Encephalitis viruses.

Desirable bacterial immunogenic compositions containing the adjuvant combinations of this invention include those directed to the prevention and/or treatment of disease caused by, without limitation, *Haemophilus influenzae* (both typable and nontypable), *Haemophilus somnus*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus faecalis*, *Helicobacter pylori*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia* psittaci, *Bordetella pertussis*, *Alloiococcus otiditis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella choleraesuis*, *Escherichia coli*, *Shigella*, *Vibrio cholerae*, *Corynebacterium diphtheriae*, *Mycobacterium tuberculosis*, *Mycobacterium avium-Mycobacterium intracellulare* complex, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Clostridium tetani*, *Leptospira interrogans*, *Borrelia burgdorferi*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Mycoplasma gallisepticum*.

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Desirable immunogenic compositions against
fungal pathogens containing the adjuvant combinations
of this invention include those directed to the
prevention and/or treatment of disease caused by,
without limitation, *Aspergillis*, *Blastomyces*, *Candida*,
Coccidiodes, *Cryptococcus* and *Histoplasma*.

· Desirable immunogenic compositions against
parasites containing the adjuvant combinations of this
invention include those directed to the prevention
and/or treatment of disease caused by, without
limitation, *Leishmania major*, *Ascaris*, *Trichuris*,
Giardia, *Schistosoma*, *Cryptosporidium*, *Trichomonas*,
Toxoplasma gondii and *Pneumocystis carinii*.

Desirable immunogenic compositions for
eliciting a therapeutic or prophylactic anti-cancer
effect in a vertebrate host, which contain the adjuvant
combinations of this invention, include those utilizing
a cancer antigen or tumor-associated antigen including,
without limitation, prostate specific antigen, carcino-
embryonic antigen, MUC-1, Her2, CA-125 and MAGE-3.

Desirable immunogenic compositions for
moderating responses to allergens in a vertebrate host,
which contain the adjuvant combinations of this
invention, include those containing an allergen or
fragment thereof. Examples of such allergens are
described in United States Patent Number 5,830,877 (51)
and published International Patent Application Number
WO 99/51259 (52), which are hereby incorporated by
reference, and include pollen, insect venoms, animal
dander, fungal spores and drugs (such as penicillin).
The immunogenic compositions interfere with the
production of IgE antibodies, a known cause of allergic
reactions.

Desirable immunogenic compositions for
moderating responses to self molecules in a vertebrate
host, which contain the adjuvant combinations of this

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invention, include those containing a self molecule or
fragment thereof.   Examples of such self molecules, in
addition to the Aβ1-42 peptide described above, include
β-chain insulin involved in diabetes, the G17 molecule

5   involved in gastroesophageal reflux disease, and
antigens which downregulate autoimmune responses in
diseases such as multiple sclerosis, lupus and
rheumatoid arthritis.
        In the case of HIV and SIV, the antigenic

10   compositions comprise at least one protein,
polypeptide, peptide or fragment derived from said
protein.   In some instances, multiple HIV or SIV
proteins, polypeptides, peptides and/or fragments are
included in the antigenic composition.

15   The adjuvant combination formulations of this
invention are also suitable for inclusion as an
adjuvant in polynucleotide immunogenic compositions
(also known as DNA immunogenic compositions).   Such
immunogenic compositions may further include

20   facilitating agents such as bupivicaine (see U.S.
Patent Number 5,593,972 (53), which is hereby
incorporated by reference).
        In order that this invention may be better
understood, the following examples are set forth.   The

25   examples are for the purpose of illustration only and
are not to be construed as limiting the scope of the
invention.

<u>Examples</u>

30

<u>Example 1</u>
<u>Materials and Methods</u>

        The following materials and methods were

35   utilized in the experiments reported in Examples 2-7
below.

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Animals

Female Balb/c mice, aged 7-9 weeks, were
purchased from Taconic Farms, Inc. (Germantown, NY).
Female Swiss-Webster mice, aged 7-9 weeks, were also
purchased from Taconic Farms, Inc. All mice were
housed in a facility approved by the American
Association for Accreditation of Laboratory Animal
Care. Mice were acclimatized to the housing facility
for one week prior to initiation of studies.

Antigens

In the HIV experiments of Examples 2-3 below,
two different synthetic peptides were used. The
sequence of the multiepitope HIV-1-$_{MN}$ peptide
T1SP10MN(A)(-Cys) (also referred to herein as MN-10) is
as follows:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:2). This peptide has been previously described
(33,34), and contains sequences from HIV-1 gp120$_{MN}$ that
evoke CD4^{+} Th cell responses in both mice and humans, a
principal neutralizing determinant, and a site
recognized by CD8^{+} CTL in Balb/c mice. The peptide was
provided by Dr. R. Scearce (Duke University, Durham,
NC). For CTL analysis, an irrelevant peptide
designated IIIB was used for comparison purposes. This
peptide corresponded to the CTL epitope within the V3
loop of HIV-1-$_{IIIB}$ (Arg Gly Pro Gly Arg Ala Phe Val Thr
Ile (SEQ ID NO:8)), and was purchased from Genosys
Biotechnologies Inc. (The Woodlands, TX). Peptides
were solubilized in sterile water, and diluted in

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appropriate buffers, or cell culture medium, prior to use.

In the amyloid experiments of Examples 4-6 and 8 below, a peptide designated Aβ1-42 was used. The sequence of Aβ1-42 is as follows:
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His
Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala
(SEQ ID NO:6).

This peptide has been previously described (47) and corresponds to an internal, 42 amino acid fragment of amyloid precursor protein. The Aβ1-42 was provided by Elan Pharmaceuticals (South San Francisco, CA). The peptide was solubilized in sterile water, and diluted in appropriate buffers, or cell culture medium, prior to use.

Adjuvants

All 529-containing adjuvant preparations were obtained from Corixa (Hamilton, MT). 529 SE was prepared as a preformulated squalene based oil-in-water (0.8-2.5% oil) emulsion, having 529 concentrations ranging from (0-50μg/ml). Aluminum phosphate was prepared in-house. Freund's complete adjuvant (CFA) and incomplete adjuvant (IFA) were purchased from Difco Laboratories, Detroit, MI. T1SP10MN(A) peptides and Freund's adjuvants were emulsified in a 1:1 ratio using two linked syringes. Recombinantly expressed murine IL-12 was provided by Genetics Institute (Cambridge, MA). Recombinant murine GM-CSF was purchased from Biosource International (Camarillo, CA) as a carrier-free lyophilized powder. Stimulon™ QS-21 was purchased from Antigenics Inc. (Framingham, MA).

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Immunizations

        Mice were immunized subcutaneously in the
rump, in a total volume of 0.2ml equally divided on
each side of the rump. Immunizations were administered
at varying time intervals, as indicated below. Antigen
and cytokines were diluted in phosphate buffered saline
to the appropriate concentrations and formulated with
adjuvants less than 16 hours prior to immunization,
under sterile conditions. Immunogenic compositions
were mixed by gentle agitation, and stored at 4°C.
Formulations were mixed by vortex immediately prior to
immunization.

Analysis of Serum Using Enzyme-linked immunosorbent
assays

        Animals were bled prior to initial
immunization, and at indicated time points. Analysis
of serum was as a geometric mean of individual titers.
For analysis of HIV peptide-specific antibody and
subclass distribution, peptide was suspended in either
carbonate buffer (15mM Na_2CO_3, 35mM $NaHCO_3$, pH 9.6), or
PBS, at a concentration of 1μg/ml and plated to 96 well
microtiter plates (Nunc) in a volume of 100:1. After
overnight incubation at 37°C, plates were washed, and
blocked (0.1% gelatin/PBS) at room temperature for 2-4
hours. ELISA plates were washed with wash buffer (PBS,
0.1% Tween™20) before addition of serially diluted
serum (PBS, 0.1% gelatin, 0.05% Tween™20, 0.02% sodium
azide). After a four hour incubation, wells were
washed and appropriate dilutions of biotinylated anti-
isotype/subclass antibodies were added for incubation
at 4°C overnight. Wells were washed and incubated with
strepavidin—conjugated horseradish peroxidase. After
incubation, wells were washed, and developed with ABTS.

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Wells were read at 405nm. Titers were standardized
using control sera.

The same protocol was followed for analysis
of Aβ1-42 peptide-specific antibody and subclass
distribution, except that a concentration of 0.3μg/ml
per microtiter plate was used.

Cell preparations

For proliferation assays and *in vitro*
cytokine analysis, spleen cells were obtained from mice
at the indicated time points. Single cell suspensions
were prepared from pools of 3-5 mice. For
proliferation and cytokine analysis, cells were
suspended in round bottom 96 well culture plates
precoated overnight with HIV peptide antigens, control
proteins, or RPMI-10 only. Spleen cells were added at
$5x10^5$ cells/well using culture medium having 2x
supplements. Cell culture supernatants were harvested
from triplicate wells for cytokine analysis three or
six days after culture initiation. Immediately after
supernatant harvest, cultures were pulsed with ^{3}H-
thymidine for 18-24 hours, and harvested to quantify
cell proliferation.

Example 2
Reciprocal anti-T1SP10MN(A)(-Cys)
IgG Endpoint Total and Subclass Titers

Reciprocal endpoint IgG subclass titers were
measured from pooled serum (n=5 Balb/c) five weeks
after initial immunization, two weeks after secondary
immunization. Mice were immunized subcutaneously in
the rump with 25μg of T1SP10MN(A)(-Cys), with a total
of 0.2ml divided equally into two 0.1ml injections on
each side, at week 0 and week 3. 529 SE was diluted to

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create an emulsion containing 1.25% squalene oil and
25μg 529 per dose. SE is an oil-in-water emulsion
vehicle consisting of squalene, glycerol, and an
emulsifying agent. Recombinant murine IL-12 was
delivered at 40ng/mouse. Recombinant murine GM-CSF was
delivered at 25μg/mouse. The results are given in
Table 1, with the geometric mean titers plus standard
errors for each group.

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Table 1
Reciprocal anti-T1SP10MN(A)(-Cys)
IgG endpoint total and subclass titers

| | | | Endpoint Titers | |
Adjuvants	μg HIV peptide	IgG	IgG1	IgG2a
529 (25) SE (1.25% oil)	25	206,301 +/- 175,149	37,567 +/- 31,526	180,671 +/- 277,211
529 (25) SE (1.25% oil) + rIL-12 (.04)	25	460,516 +/- 690,712	74,269 +/- 169,868	222,446 +/- 400,716
529 (25) SE (1.25% oil) + GM-CSF (25)	25	1,085,658 +/- 1,064,924	238,379 +/- 62,199	117,657 +/- 25,301

Example 3
CTL Analysis in Balb/c Mice

The protocols of Example 2 were followed regarding immunization of mice. The CTL activity of spleen cells isolated from mice 14 days after secondary immunization was assessed. 529 SE was formulated with 25μg 529 SE containing 1.25% oil, with or without 10μg GM-CSF or 40ng IL-12, plus 25μg T1SP10MN(A)(-Cys).

For CTL analysis, spleen cells were removed from immunized mice 14 days after secondary immunization. A protocol previously described (39) was essentially followed. Briefly, erythrocyte-depleted spleen cells from three mice per group were pooled. Spleen effector cells ($4x10^6$/ml) were restimulated in 24 well culture plates in a volume of 1.5-2 ml for

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seven days with 1μg/ml of either the "MN-10" peptide, the "IIIB" 10mer CTL epitope peptide, or no HIV peptide. Both CTL epitopes were restricted to H-2D^d. Cultures were supplemented with 10U/ml recombinant murine IL-2 (Biosource) for the last five days of culture. For analysis of cytotoxic activity, P815 cells were labeled with Cr^{51} and pulsed with 5μg/ml peptide (IIIB or MN-10) for four hours, and added to cultured splenic effector cells. When no HIV peptide was used, that set of target cells was not pulsed. Three-fold dilutions of effector to target cell ratios ("E:T") were used, from 30:1 through 1.1:1. Percent CTL activity was calculated as the percentage of chromium release using ((specific chromium release – spontaneous chromium release) / (maximal chromium release – spontaneous chromium release)) x 100. Chromium release was assessed after a six hour incubation period. The average spontaneous release of chromium was always less than 15% of maximal release. The results of data from day 28 are shown in Table 2.

Table 2
Effector to Target Ratios

529 SE+:

E:T% spec- ific release	Peptide MN-10			Peptide IIIB			No Peptide		
	*	IL-12	GM-CSF	*	IL-12	GM-CSF	*	IL-12	GM-CSF
30:1	31	53	71	8	11	19	4	8	8
10:1	19	41	70	5	8	21	2	5	9
3.3:1	5	11	35	2	1	5	-2	-2	-1
1.1:1	2	4	14	1	0	2	-3	-2	-3

* No additional adjuvant

Example 4
Reciprocal anti-Aβ1-42 IgG Endpoint
Total and Subclass Titers

Outbred Swiss-Webster mice were divided into
groups of ten mice each. Each group received 30μg of
Aβ1-42 peptide, which corresponds to an internal 42
amino acid long region of APP. The first group did not
receive an adjuvant; the second group received 50μg of
529 SE containing 2.5% oil ; the third group received
50μg of 529 SE containing 2.5% oil plus 10μg GM-CSF; the
fourth group received 10μg GM-CSF; the fifth group
received SE containing 1.25% oil; the sixth group
received SE containing 1.25% oil plus 10μg GM-CSF; the
seventh group received 50μg QS-21. Mice were immunized
subcutaneously in the rump with a total volume of 0.2ml,
divided equally into each of two sites at the base of
the tail/rump. Immunizations were administered at week
0 and week 3.

Mice were bled at days 0, 20, 35 and 70.
Serum was analyzed from individual mice. Reciprocal

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endpoint anti-Aβ1-42 peptide IgG endpoint total class
and subclass titers were measured from individual sera
(n=10 Swiss-Webster) at week 5 and at week 10. The IgG
endpoint results are given in Tables 3 (week 5) and 4
5   (week 10). The IgG1 subclass results are given in
Tables 5 (week 5; the groups receiving no adjuvant or
QS-21 were not measured) and 6 (week 10). The IgG2a
subclass results are given in Tables 7 (week 5; the
groups receiving no adjuvant or QS-21 were not measured)
10   and 8 (week 10).

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Table 3

Anti-Aβ1-42 Week 5 IgG Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
None	12,976	+/- 14,386
529 SE (50)	16,204	+/- 225,221
529 SE (50) + GM-CSF (10)	608,474	+/- 623,575
GM-CSF (10)	214,497	+/- 609,067
SE (1%)	33,342	+/- 15,493
SE (1%) + GM-CSF (10)	453,367	+/- 162,750
QS-21 (50)	4,076	+/- 9,036

Table 4

Anti-Aβ1-42 Week 10 IgG Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
None	21,426	+/- 24,959
529 SE (50)	86,847	+/- 187,792
529 SE (50) + GM-CSF (10)	943,075	+/- 989,177
GM-CSF (10)	1,049,414	+/- 390,525
SE (1%)	255,631	+/- 114,025
SE (1%) + GM-CSF (10)	1,005,899	+/- 407,108
QS-21 (50)	47,222	+/- 159,775

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Table 5

Anti-Aβ1-42 Week 5 IgG1 Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (50)	461	+/- 627
529 SE (50) + GM-CSF (10)	1,936	+/- 12,680
GM-CSF (10)	8,654	+/- 10,100
SE (1%)	4,515	+/- 6,273
SE (1%) + GM-CSF (10)	24,422	+/- 19,764

5

Table 6

Anti-Aβ1-42 Week 10 IgG1 Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
None	2,086	+/- 2,448
529 SE (50)	969	+/- 521
529 SE (50) + GM-CSF (10)	2,076	+/- 4,901
GM-CSF (10)	8,483	+/- 10,998
SE (1%)	3,623	+/- 3,456
SE (1%) + GM-CSF (10)	12,472	+/- 11,502
QS-21 (50)	988	+/- 895

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Table 7

Anti-Aβ1-42 Week 5 IgG2a Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (50)	2,224	+/- 10,099
529 SE (50) + GM-CSF (10)	94,764	+/- 849,173
GM-CSF (10)	25,554	+/- 13,191
SE (1%)	1,484	+/- 2,271
SE (1%) + GM-CSF (10)	8,405	+/- 31,303

5

Table 8

Anti-Aβ1-42 Week 10 IgG2a Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
None	5,910	+/- 39,626
529 SE (50)	5,944	+/- 9,100
529 SE (50) + GM-CSF (10)	47,694	+/- 88,053
GM-CSF (10)	64,910	+/- 54,824
SE (1%)	2,350	+/- 2,326
SE (1%) + GM-CSF (10)	7,421	+/- 31,153
QS-21 (50)	3,544	+/- 26,332

10

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Example 5
Reciprocal anti-Aβ1-42 Endpoint Total and
Subclass Titers with Varying Amounts of GM-CSF

5   Outbred Swiss-Webster mice were divided into
groups of ten mice each. Each group received two
immunizations of 30μg of Aβ1-42 peptide at weeks 0 and
3. The first group received 25μg of 529 SE plus 10μg
GM-CSF; the second group received 25μg of 529 SE plus
10   1μg GM-CSF; the third group received 25μg of 529 SE plus
0.1μg GM-CSF; the fourth group received 25μg of 529 SE
plus 10μg GM-CSF in the priming dose, followed by 25μg
529 SE only in the second dose; the fifth group received
25μg QS-21; the sixth group received 25μg QS-21 plus
15   10μg GM-CSF. Mice were immunized subcutaneously in the
rump with a total volume of 0.2ml, divided equally into
each of two sites at the base of the tail/rump.
          Mice were bled at days 0, 21 and 42.
Reciprocal endpoint anti-Aβ1-42 peptide IgG class and
20   subclass titers were measured from individual serum
(n=10) at week 6. The IgG endpoint results are given in
Table 9. The IgG1 subclass results are given in Table
10. The IgG2a subclass results are given in Table 11.

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Table 9

Anti-Aβ1-42 Week 6 IgG Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (25) + GM-CSF (10)	353,660	+/- 148,940
529 SE (25) + GM-CSF (1)	150,935	+/- 218,332
529 SE (25) + GM-CSF (0.1)	86,145	+/- 91,724
529 SE (25)*	25,365	+/- 54,083
QS-21 (25)	1,866	+/- 18,430
QS-21 (25) + GM-CSF (10)	48,970	+/- 116,106

* First dose 529 SE (25) + GM-CSF (10); second dose
  529 SE (25) alone

Table 10

Anti-Aβ1-42 Week 6 IgG1 Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (25) + GM-CSF (10)	10,867	+/- 18,333
529 SE (25) + GM-CSF (1)	24,909	+/- 18,625
529 SE (25) + GM-CSF (0.1)	6,608	+/- 17,736
529 SE (25)*	4,511	+/- 8,154
QS-21 (25)	581	+/- 126
QS-21 (25) + GM-CSF (10)	7,618	+/- 29,145

* First dose 529 SE (25) + GM-CSF (10); second dose
  529 SE (25) alone

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Table 11

Anti-Aβ1-42 Week 6 IgG2a Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (25) + GM-CSF (10)	243,758	+/- 354,383
529 SE (25) + GM-CSF (1)	116,222	+/- 143,140
529 SE (25) + GM-CSF (0.1)	98,018	+/- 391,797
529 SE (25)*	16,018	+/- 165,298
QS-21 (25)	not done	+/- not done
QS-21 (25) + GM-CSF (10)	30,133	+/- 134,774

* First dose 529 SE (25) + GM-CSF (10); second dose
  529 SE (25) alone

Example 6

Reciprocal anti-Aβ1-42 Endpoint Total and
Subclass Titers with Varying Amounts of GM-CSF

Outbred Swiss-Webster mice were divided into
groups of ten mice each. Each group received
immunizations at week 0 and at week 3, with 30μg of Aβ1-
42 peptide each time. In the week 0 immunization, the
first group received 50μg of 529 SE; the second group
received 50μg of 529 SE plus 10μg GM-CSF; the third
group received 50μg of 529 SE plus 5μg GM-CSF; the
fourth group received 50μg of 529 SE plus 2μg GM-CSF;
the fifth group received 50μg of 529 SE plus 0.5μg GM-
CSF; the sixth group received 1% SE. In the week 3
immunization, the first through fifth groups received
the same dose as the week 0 immunization, except that
the 529 SE was reduced from 50 to 25μg. The amount of
SE received by the sixth group was increased from 1% in
the week 0 immunization to 1.2% in the week 3
immunization. Mice were immunized subcutaneously in the

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rump with a total volume of 0.2ml, divided equally into each of two sites at the base of the tail/rump.

Mice were bled at days 2, 20 and 35. Reciprocal endpoint anti-Aβ1-42 peptide IgG class and subclass titers were measured from individual serum (n=10) at week 5. The IgG endpoint results are given in Table 12. The IgG1 subclass results are given in Table 13. The IgG2a subclass results are given in Table 14.

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Table 12
Anti-Aβ1-42 Week 5 IgG Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (50)	6,119	+/- 3,103
529 SE (50) + GM-CSF (10)	52,312	+/- 78,421
529 SE (50) + GM-CSF (5)	16,392	+/- 17,706
529 SE (50) + GM-CSF (2)	321,524	+/- 224,875
529 SE (50) + GM-CSF (0.5)	36,934	+/- 29,449
SE (1%)	7,784	+/- 9,041

5

Table 13
Anti-Aβ1-42 Week 5 IgG1 Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (50)	499	+/- 676
529 SE (50) + GM-CSF (10)	1,424	+/- 2,468
529 SE (50) + GM-CSF (5)	3,407	+/- 5,653
529 SE (50) + GM-CSF (2)	18,328	+/- 8,067
529 SE (50) + GM-CSF (0.5)	3,526	+/- 17,606
SE (1%)	2,556	+/- 5,615

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Table 14

Anti-Aβ1-42 Week 5 IgG2a Endpoint Titers

Adjuvants	Geometric Mean	Standard Error
529 SE (50)	1,386	+/- 5,173
529 SE (50) + GM-CSF (10)	48,519	+/- 148,981
529 SE (50) + GM-CSF (5)	11,659	+/- 23,132
529 SE (50) + GM-CSF (2)	124,815	+/- 167,340
529 SE (50) + GM-CSF (0.5)	26,190	+/- 40,254
SE (1%)	694	+/- 1,325

5   Example 7

Th-CTL and C4-V3 Peptide Immunization of Rhesus Monkeys

          The following experiment was designed to
     directly compare a number of peptide and adjuvant
10   combination formulations in a primate species (rhesus
     monkeys) in order to identify potential peptide/adjuvant
     combinations to move forward into human clinical trials.
     Specifically, the adjuvant formulation 529 SE with human
     GM-CSF was evaluated in comparison to incomplete
15   Freund's adjuvant (IFA) in combination with (1) an SIV
     env-derived T-helper/SIV gag CTL peptide conjugate (ST1-
     p11C) having the following sequence:
               Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly
               Lys Asn Val Tyr Leu Glu Gly Cys Thr Pro Tyr
20   Asp Ile Asn Gln Met Leu (SEQ ID NO:3);
     or (2) an HIV-1 derived C4-V3 peptide conjugate (C4-
     V3$_{89.6P}$) having the following sequence:
               Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly
               Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn

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Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg
Ala Phe Tyr Ala Arg Arg (SEQ ID NO:4).

Study Design: A total of 8 animals was used
for study, 4 Mamu-A*01+ and 4 Mamu-A*01- as described in
Table 15.

Table 15
529 SE & GM-CSF vs. IFA

Group #	# Animals	Animal	Immunogenic composition	Adjuvant
1	2 Mamu-A01+	95X009 93X021	ST1-p11C	IFA
2	2 Mamu-A01+	98N002 98N008	ST1-p11C	529 SE/GM-CSF
3	2 Mamu-A01-	98N007 98N013	C4-V3$_{89.6P}$	IFA
4	2 Mamu-A01-	95X011 96X004	C4-V3$_{89.6P}$	529 SE/GM-CSF

Group 1 animals received 0.5ml of the Th-CTL
peptide ST1-p11C (1.0 mg/ml) in a water in oil emulsion
with 0.5ml IFA in a total volume of 1.0 ml. The group 2
animals received 0.5ml of ST1-p11C (1.0 mg/ml) combined
with 250μg of human GM-CSF, 50μg of 529 SE with a final
oil concentration of 1% in a total volume of 1.0ml.
Group 3 animals received 0.5ml of the C4-V3$_{89.6P}$ peptide
(2.0 mg/ml) in a water in oil emulsion with 0.5ml of
IFA, final volume of 1.0ml. Finally the group 4 animals
received 0.5ml C4-V3$_{89.6P}$ peptide (2.0 mg/ml) combined
with 250μg of human GM-CSF, 50μg of 529 SE with a final
oil concentration of 1% in a total volume of 1.0 ml.
    All animals were immunized by intramuscular
injection on a schedule of 0, 4, and 8 weeks.
Peripheral blood samples were drawn immediately before
and 1 or 2 weeks after each immunization to monitor CTL

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induction by tetramer staining, p11C (Cys Thr Pro Tyr Asp Ile Asn Gln Met; SEQ ID NO:3, amino acids 19-27)-specific ELISPOT responses and bulk culture CTL responses (Groups 1 and 2) as well as for peptide specific antibody responses (Groups 1-4).

Safety and Tolerability

ST1-p11C + IFA: The ST1-p11C + IFA formulation which was administered by intramuscular injection at a single site three times in the group 1 animals was associated with significant injection site reactivity. One animal (93x021) developed a 1.5 cm sized abscess at the site of injection two weeks after the second immunization. The other animal (95x009) also developed a 2.0 cm sized abscess at the site of injection two weeks after the third immunization which broke through the skin and required dressing.

ST1-p11C + 529 SE/GM-CSF: The ST1-p11C + 529 SE/GM-CSF formulation which was administered by intramuscular injection at a single site three times in the group 2 animals was associated with minor adverse effects. Both of the group 2 animals vomited shortly after receiving the third immunization at week 8. No other adverse effects were noted.

C4-V3$_{89.6P}$ + IFA: The C4-V3$_{89.6P}$ + IFA formulation which was administered by intramuscular injection at a single site three times in the group 3 animals was associated with significant injection site reactivity. One animal (98n013) developed a 1.0 cm sized abscess at the site of injection one week after the second immunization. The other animal (98n007) also developed a 1.5 cm sized abscess at the site of injection one week after the second immunization. This animal's abscess required draining and dressing four weeks later.

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C4-V3$_{89.6P}$ + 529 SE/GM-CSF: The C4-V3$_{89.6P}$ + 529
SE/GM-CSF formulation which was administered by
intramuscular injection at a single site three times in
the group 4 animals was associated with one minor
adverse effect.   One of the group 4 animals (95x011)
vomited shortly after receiving the third immunization
at week 8.   No other adverse effects were noted.

In all of the group 1 and group 3 animals in
which the animals received IFA as the adjuvant,
significant injection site reactivities were seen.
These results indicate that 0.5mls of IFA is poorly
tolerated when given by intramuscular injection at a
single injection site.   It is also worth noting that 3
of the 4 animals that received 529 SE/GM-CSF as the
adjuvant at week 8 vomited shortly after being
immunized.   While the anesthetic used (ketamine) is
known to be associated with vomiting, no other cases of
animals vomiting were documented over the course of the
study.   At this time, insufficient evidence exists to
attribute the animal's vomiting to any adverse effects
associated with 529 SE/GM-CSF.

Results: Induction of Cellular Immune Responses (Groups
1 and 2)

Fresh Blood p11C-tetramer Staining: Prior to
immunization, and one and two weeks post-immunization,
freshly isolated peripheral blood from all the Mamu-A*01
positive animals (Groups 1 and 2) was screened for the
presence of p11C-specific CD3$^+$CD8$^+$ T lymphocytes by
soluble MHC Class I tetramer staining.   As shown in
Table 16, only one animal (93x021) which received the
ST1-p11C peptide in combination with IFA, showed
evidence of immunization-induced cellular immune
responses in unstimulated peripheral blood.

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Table 16

Percent p11C-tetramer staining and p11C-specific ELISPOT responses in freshly isolated peripheral blood

Animal/ (Group)	Formulation	Week 0 Pre-immunization		Week 5 1 wk post 2nd immunization		Week 6 2 wks post 2nd immunization	
		Fresh Bld Tetramer staining[a]	ELISPOT # SFC Per 10^6 cells	Fresh Bld Tetramer staining	ELISPOT # SFC Per 10^6 cells	Fresh Bld Tetramer staining	ELISPOT # SFC Per 10^6 cells
95x009(1)	ST1-p11C + IFA	0.02	0.0	0.00	0.0	0.00	3.8
93x021(1)	ST1-p11C + IFA	0.02	Nd[b]	0.15	56.3	0.12	21.9
98n002(2)	ST1-p11C + 529SE/ GM-CSF	0.00	0.6	0.05	0.0	0.01	6.3
98n008(2)	ST1-p11C + 529SE/ GM-CSF	0.05	0.0	0.04	15.0	0.01	9.4

Animal	Formulation	Week 8 4 wks post 2nd immunization		Week 9 1 wk post 3rd immunization		Week 10 2 wks post 3rd immunization	
		Fresh Bld	ELISPOT	Fresh Bld	ELISPOT	Fresh Bld	ELISPOT
95x009(1)	ST1-p11C + IFA	0.00	Nd	0.01	2.5	0.02	1.9
93x021(1)	ST1-p11C + IFA	0.02	Nd	0.14	Nd	0.02	Nd
98n002(2)	ST1-p11C + 529SE/ GM-CSF	0.06	Nd	0.00	Nd	0.00	3.8
98n008(2)	ST1-p11C + 529SE/ GM-CSF	0.02	Nd	0.02	Nd	0.00	0.0

[a] Reported as the percentage of fresh blood $CD3^+CD8^+$ lymphocytes staining positive with the p11C-tetramer; ND, not done.
[b] Nd = No data (in this and subsequent Tables).

P11C-specific ELISPOT responses: To further evaluate the induction of cellular immune responses in

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the Group 1 and 2 animals, freshly isolated peripheral
blood lymphocytes were screened for the presence of
p11C-specific CD3⁺CD8⁺ T lymphocytes by ELISPOT
analysis. As shown in Table 16, only animal 93x021,
5   which demonstrated detectable levels of p11C-specific
CD8⁺ lymphocytes by fresh blood tetramer analysis, had
detectable p11C-specific CD8⁺ T lymphocytes by ELISPOT
analysis. In every case, a positive response by p11C-
tetramer analysis was corroborated by a positive p11C-
10   specific ELISPOT response.

        p11C-specific cellular immune responses after
in vitro peptide p11C stimulation: In an effort to
increase the number of p11C-specific cells prior to
15   analysis, freshly isolated peripheral blood lymphocytes
were stimulated *in vitro* with peptide p11C and rhIL-2.
After 14 days, the resulting effector cells were
screened for p11C-tetramer binding as well as for
functional p11C-specific lytic activity in a standard
20   chromium release CTL assay. The results of the p11C-
tetramer analysis and the functional CTL assays are
shown in Table 17. Animal 93x021, which consistently
showed p11C-specific immune responses in freshly
isolated lymphocytes, demonstrated a very high level of
25   tetramer binding and functional CTL activity one week
after the second immunization. This indicated the
induction of a very potent p11C-specific cellular immune
response. In contrast to the results seen in the
freshly isolated lymphocytes, one animal from Group 2
30   (98n008, ST1-p11C + 529 SE/GM-CSF) began to show
evidence of p11C-specific cellular immune responses two
weeks after the second immunization. However, the p11C-
specific immune response seen in the Group 2 animal was
of a significantly lower magnitude than that seen in the
35   responding animal from Group 1.

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Table 17

Percent p11C-tetramer staining and functional p11C-specific CTL responses after *in vitro* peptide p11C stimulation

Animal/ Group	Formul- ation	Week 0 Pre-immunization		Week 5 1 wk post 2nd immunization		Week 6 2 wks post 2nd immunization	
		Tetramer staining[a]	CTL 20:1 E:T[b]	Tetramer staining	CTL 20:1 E:T	Tetramer staining	CTL 20:1 E:T
95x009(1)	ST1-p11C + IFA	0.03	0.6	0.23	Nd	0.27	0.0
93x021(1)	ST1-p11C + IFA	0.03	0.0	34.31	66.3	15.15	4.69
98n002(2)	ST1-p11C + 529SE/ GM-CSF	0.21	0.0	Nd	Nd	0.73	Nd
98n008(2)	ST1-p11C + 529SE/ GM-CSF	0.16	0.0	Nd	Nd	5.84	Nd

Animal/ Group	Formul- ation	Week 8 4 wks post 2nd immunization		Week 9 1 wk post 3rd immunization		Week 10 2 wks post 3rd immunization	
		Tetramer Staining	CTL 20:1 E:T	Tetramer Staining	CTL 20:1 E:T	Tetramer Staining	CTL 20:1 E:T
95x009(1)	ST1-p11C + IFA	Nd	Nd	0.76	3.0	0.72	0.0
93x021(1)	ST1-p11C + IFA	Nd	Nd	4.90	7.3	Nd	Nd
98n002(2)	ST1-p11C + 529SE/ GM-CSF	Nd	Nd	0.07	5.7	0.39	0.0
98n008(2)	ST1-p11C + 529SE/ GM-CSF	Nd	Nd	2.24	11.4	5.00	0.0

[a] Reported as the percentage of CD3+CD8+ cultured cells staining positive with the p11C-tetramer.
[b] Reported as the percent p11C-specific lysis (minus background) at an effector to target ratio (E:T) of 20:1.

Intracellular Cytokine Analysis: To further characterize the functional and phenotypic properties of the immunogen-induced peptide p11C-specific lymphocytes, the intracellular expression was monitored of the Th1

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type cytokines INF-γ, TNFα, IL-2 and the Th2 type
cytokine IL-4. Intracellular cytokine expression was
monitored in peripheral blood lymphocytes after an
initial *in vitro* stimulation in the presence of 10μM
5   peptide p11C and rhIL-2. The cultures were then
maintained for 14 days with 40U/ml IL-2. After two
weeks, the cultured cells were stimulated with either
media alone, or with 10μM peptide p11C + anti-human CD28
and anti-human CD49d for one hour. After one hour, the
10   cells were treated with Brefeldin A for an additional
five hours to allow for intracellular cytokines to
concentrate in the endoplasmic reticulum. Intracellular
cytokine expression was then quantitated by flow
cytometry (Tables 18 & 19).

15   As shown in Table 18, two weeks after *in vitro*
peptide p11C stimulation, CD3$^+$ peripheral blood
lymphocytes from the group 1 animal 93x021 (ST1-p11C +
IFA), demonstrated a low level of Th1 type cytokine
expression, with less than 1.5% of all cells actively
20   secreting INF-γ, TNFα, or IL-2. Approximately 8% of all
CD3$^+$ lymphocytes after *in vitro* peptide p11C stimulation
were actively secreting the Th2 type cytokine IL-4, and
the IL-4 secreting cells were found to be limited to the
CD3$^+$CD4$^+$ lymphocyte subset. After a brief re-exposure
25   to the peptide p11C, the p11C-tetramer$^+$ and CD3$^+$CD8$^+$
lymphocyte subsets were actively secreting the Th1 type
cytokines INF-γ and TNFα, but could not be induced to
secrete significantly increased levels of IL-2. After
peptide p11C re-exposure, the secretion of the Th2 type
30   cytokine IL-4 was unaffected.

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Table 18

Intracellular cytokine analysis, group 1 animal 93x021, two weeks post second immunization

Lymphocyte Subset	Media	INF-γ p11C	[a]S.I.	Media	TNFα p11C	S.I.
[b]P11C-tetramer^{+}	977	27,612	28.3	90	20,342	226.0
[b]Bulk CD3^{+}	7,628	65,567	8.6	12,256	51,361	4.2
[b]CD3^{+}CD4^{+}	2,488	5,545	2.2	6,252	2,827	0.4
[b]CD3^{+}CD8^{+}	5,273	60,573	11.5	6,865	48,439	7.1

	Media	IL-2 p11C	S.I.	Media	IL-4 p11C	S.I.
[b]P11C-tetramer^{+}	751	2,004	2.7	Nd	Nd	Nd
[b]Bulk CD3^{+}	2,879	6,463	2.2	78,069	90,563	1.2
[b]CD3^{+}CD4^{+}	1,377	1,683	1.2	71,275	81,437	1.1
[b]CD3^{+}CD8^{+}	1,502	4,783	3.2	6,794	9,126	1.3

Table 19

Intracellular cytokine analysis, group 2 animal 98n008, one week post third immunization

Lymphocyte Subset	Media	INF-γ p11C	[a]S.I.	Media	TNFα p11C	S.I.
[b]P11C-tetramer^{+}	545	560	1.0	748	454	0.0
[b]Bulk CD3^{+}	6,829	10,489	1.5	3,402	13,443	4.0
[b]CD3^{+}CD4^{+}	3,310	3,789	1.1	1,428	2,567	1.8
[b]CD3^{+}CD8^{+}	3,189	6,825	2.1	2,587	11,324	4.4

	Media	IL-2 p11C	S.I.	Media	IL-4 p11C	S.I.
[b]P11C-tetramer^{+}	77	456	5.9	nd	nd	nd
[b]Bulk CD3^{+}	385	2,868	7.4	219,789	202,122	0.9
[b]CD3^{+}CD4^{+}	1,379	1,761	1.3	170,804	158,175	0.9
[b]CD3^{+}CD8^{+}	36	2,095	58.2	49,053	44,083	0.9

[a]   S.I., Stimulation index.
[b]   reported as the number of indicated cells staining positive for the indicated cytokine per 10^6 CD3^{+} cells, minus background (isotype control) staining.

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As shown in Table 19, two weeks after *in vitro* peptide p11C stimulation, CD3$^+$ peripheral blood lymphocytes from the group 2 animal 98n008 (ST1-p11C + 529 SE/GM-CSF) demonstrated a low level of Th1 type cytokine expression, with less than 1.0% of all cells actively secreting INF-γ, TNFα, or IL-2. Interestingly, approximately 20% of all CD3$^+$ lymphocytes were actively secreting the Th2 type cytokine IL-4, a 2.5 fold increase in the number of IL-4 producing cells as compared to the Group 1 animal. As was the case with the Group 1 animal, the IL-4 secreting cells were found to be limited to the CD3$^+$CD4$^+$ lymphocyte subset. After a brief re-exposure to the peptide p11C, the p11C-tetramer$^+$ and CD3$^+$CD8$^+$ lymphocyte subsets from the group 2 animal could be stimulated to secrete TNFα, but not INF-γ. In contrast to the Group 1 animal, after peptide re-exposure, a significant increase in IL-2 expression by CD3$^+$CD8$^+$ cells could be demonstrated. As was the case with the Group 1 animal, following the re-exposure to the peptide p11C, the secretion of the Th2 type cytokine IL-4 was unchanged.

Results: Immunogen-Induced Humoral Immune Responses (Groups 1 and 2):

To evaluate the induction of immunogen-specific humoral antibody responses, the anti-ST1-p11C ELISA antibody titers were measured in the serum of the group 1 and 2 animals immediately prior to immunization and 1 and 2 weeks after the second and third immunizations. The results are summarized in Table 20.

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Table 20
ELISA end-point titers of serum from rhesus monkeys
immunized with ST1-p11C (groups 1 and 2)

Animal	Group	Formulation	Week	ST1-p11C Ab titer[a]
95x009	1	ST1-p11C + IFA	5	12,800
			6	12,800
			9	6,400
			10	12,800
93x021	1	ST1-p11C + IFA	5	51,200
			6	102,400
			9	51,200
			10	51,200
98x002	2	ST1-p11C + 529SE/GM-CSF	5	<50
			6	<50
			9	1,600
			10	<50
98n008	2	ST1-p11C + 529SE/GM-CSF	5	200
			6	200
			9	12,800
			10	6,400

[a] Antibody end-point binding titers were determined as the
reciprocal of the highest dilution of the plasma giving an
OD reading of experimental/control (E/C) of > 3.0.

Immunogen Induced Humoral Immune Responses (Groups 3 and 4):

To evaluate the induction of immunogen-
specific and adjuvant-specific humoral antibody
responses, the anti-C4-V3$_{89.6P}$ and anti-GM-CSF ELISA
antibody titers were measured in the plasma of the group
3 and 4 animals immediately prior to immunization and
one and two weeks after the second and third
immunizations. The results are summarized in Table 21.
The results indicate that peak plasma C4-V3$_{89.6P}$ antibody

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titers were generated at one week after the second
immunization in all the animals tested. Peak plasma
antibody titers in the group 3 animals (C4-V3$_{89.6P}$ + IFA)
were several orders of magnitude higher than the peak
plasma antibody titers seen in the group 4 (C4-V3$_{89.6P}$ +
529 SE/GM-CSF). The group 4 animals demonstrated low
but detectable levels of anti-GM-CSF antibody titer that
peaked one week after the third immunization.

Table 21
ELISA end-point titers of plasma from rhesus monkeys immunized with C4-V3$_{89.6P}$ (groups 3 and 4)

Animal/ Group	Formulation	Week	C4-V3 Ab titer[a]	Anti-human GM-CSF Ab titer
98n007(3)	C4-V3$_{89.6P}$ + IFA	5	102,400	<10
		6	25,600	<10
		9	25,600	<10
		10	25,600	<10
98n013(3)	C4-V3$_{89.6P}$ + IFA	5	12,800	<10
		6	6,400	<10
		9	12,800	10
		10	12,800	<10
96x004(4)	C4-V3$_{89.6P}$ + 529SE/GM-CSF	5	6,400	320
		6	1,600	160
		9	3,200	2,560
		10	1,600	1,280
95x011(4)	C4-V3$_{89.6P}$ + 529SE/GM-CSF	5	1,600	160
		6	800	80
		9	1,600	1,280
		10	1,600	1,280

[a] Antibody end-point binding titers were determined as the
reciprocal of the highest dilution of the plasma giving an OD
reading of experimental/control (E/C) of > 3.0.

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The induction of neutralizing antibodies in the group 3 and 4 animals was also monitored; the results are summarized in Table 22. The results indicate that both group 3 animals and both group 4 animals had developed neutralizing antibodies that were capable of neutralizing the $SHIV_{89.6}$ virus *in vitro*. The $SHIV_{89.6}$ neutralizing antibody titers seen in the group 3 animals were generally higher than that seen in the group 4 animals. In addition, after the final immunization, the serum from the group three animals demonstrated a low level of neutralizing activity against the $SHIV_{89.6P}$ strain of the virus, which is difficult to neutralize.

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Table 22

Serum end-point neutralizing antibody titers from rhesus monkeys immunized with C4-V3$_{89.6P}$ (groups 3 and 4)

			Neutralizing antibody to[a]	
Animal/ Group	Formulation	Week	SHIV$_{89.6}$	SHIV$_{89.6P}$
98n007(3)	C4-V3$_{89.6P}$ + IFA	0	<10	<10
		5	22	<10
		6	46	<10
		9	113	46
		10	74	71
98n013(3)	C4-V3$_{89.6P}$ + IFA	0	<10	<10
		5	12	<10
		6	19	<10
		9	36	18
		10	22	<10
96x004(4)	C4-V3$_{89.6P}$ + 529SE/GM-CSF	0	<10	<10
		5	<10	<10
		6	<10	<10
		9	26	<10
		10	<10	<10
95x011(4)	C4-V3$_{89.6P}$ + 529SE/GM-CSF	0	<10	<10
		5	11	<10
		6	<10	<10
		9	19	<10
		10	<10	<10

5

[a] Neutralizing antibody titers are the reciprocal serum dilution at which 50% of cells were protected from virus-induced killing as measured by neutral red uptake.

10

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Example 8
Therapeutic Efficacy Study of PDAPP Transgenic Mice
Treated with Aβ1-42 and Adjuvant(s)

The following experiment was designed to compare a number of adjuvant combination formulations in PDAPP transgenic mice to test the therapeutic efficacy of the Aβ1-42 peptide.

Study Design: Ten and a half to twelve and a half month old PDAPP transgenic mice (males and females) were divided into four groups of 40 mice, sorted such that each group was matched to each other as closely as possible for age, sex and transgenic parent. The groups were as described in Table 23:

Table 23
Transgenic Mice Treatment Groups

Group	Adjuvant	Dose	Aβ1-42 Dose	N at Start	N at End
1	MPL SE	25 μg	75/60 μg	40	35
2	MPL SE+GM-CSF	25 μg/10 μg	64/60 μg	41	34
3	529+GM-CSF	25 μg/10 μg	64/60 μg	40	31
4	PBS	na	na	40	37

Aβ1-42 peptide was from Elan Pharmaceuticals, 529 SE and MPL™ SE were from Corixa, and murine GM-CSF was from Biosource. All mice received injections at weeks 0, 2, 4, 8, 12, 16, 20 and 24. Mice were bled 5-7 days post-injection, starting after the second injection. Groups 1, 2, and 3 were injected subcutaneously with a volume of 200 μl, while group 4 received 250 μl subcutaneous dosing. Animals were sacrificed at week 25 of the Study. Titers were obtained using a dilution which gives a value of 50% of the maximum optical density reading.

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Results

Immunogenicity and Antibody Response: All
groups reached their peak geometric mean titer (GMT) of
antibodies to the Aβ1-42 peptide at either the second
(RC529 + GM-CSF) or third bleed (MPL™ SE, MPL™ SE + GM-
CSF) (see Figure 1). At peak GMT, MPL™ SE + GM-CSF was
16,400, while 529 SE + GM-CSF was 13,400 or
approximately 1.5 times the MPL™ SE control of 9,700.
However, the titers on the two GM-CSF-containing
formulations (Groups 2 and 3) fell back to the
approximate level of the MPL™ SE control, with final
GMTs of MPL™ SE = 4600, MPL™ SE + GM-CSF = 5350, 529 SE
+ GM-CSF = 4650.

To determine if the eventual decrease in titer
of the two GM-CSF-containing formulations was due to an
anti-GM-CSF antibody response, an ELISA was used to
determine if anti-GM-CSF antibodies had formed over the
course of the immunization. Sera from all animals
receiving GM-CSF were titered against the murine GM-CSF
used throughout this experiment. No evidence of anti-
GM-CSF antibodies was found in any of the treated
animals (data not shown).

Brain Aβ Levels: All three treatment groups
significantly reduced the accumulation of both total Aβ
peptide (Figure 2-individual results; Table 24-pooled
results) and Aβ1-42 (Figure 3-individual results; Table
25-pooled results) in the cortical region of the PDAPP
mouse brain. Aβ ELISAs (total and 1-42 forms) were
performed as previously described (54) using guanidine-
solubilized brain homogenates. Statistical comparisons
used the Mann-Whitney test of significance.

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Table 24
Cortical Total Aβ Levels

	PBS	MPL SE	MPL SE + GM-CSF	529 SE + GM-CSF
Median (ng/g)	6,478	1,707	925	1,313
Range	64 – 17,208	33 – 8,501	67 – 5,293	79 – 5,271
% Reduction	---	74	86	80
P Value (M-W)	---	<0.0001	<0.0001	<0.0001
N	37	35	34	31

Table 25
Cortical Aβ1-42 Levels

	PBS	MPL SE	MPL SE + GM-CSF	529 SE + GM-CSF
Median (ng/g)	5,609	1,799	779	1,127
Range	284 – 14,004	10 – 6,715	43 – 4,824	29 – 4,442
% Reduction	---	68	86	80
P Value (M-W)	---	<0.0001	<0.0001	<0.0001
N	36	35	34	31

Amyloid Burden: The extent of amyloidosis was quantified in the frontal cortex using immunohistochemical methods as previously described (55). All three treatment groups showed a significant reduction in amyloid burden (Figure 4-individual results; Table 26-pooled results).

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Table 26
Frontal Cortex Amyloid Burden

	PBS	MPL SE	MPL SE + GM-CSF	529 SE + GM-CSF
Median (%AB)	7.98	0.49	0.00	0.04
Range	0.00 - 27.37	0.00 - 9.63	0.00 - .83	0.00 - 5.53
% Reduction	------	94	100	99.5
P Value (M-W)	------	<0.0001	<0.0001	<0.0001
N	29	33	29	30

Neuritic Burden: The effect of treatment on
the development of neuritic dystrophy in the frontal
cortex was assessed immunohistochemically as previously
described (55). All three treatment groups
significantly reduced the extent of the neuritic burden
relative to PBS control (Figure 5-individual results;
Table 27-pooled results).

Table 27
Frontal Cortex Neuritic Burden

	PBS	MPL SE	MPL SE + GM-CSF	529 SE + GM-CSF
Median (%NB)	0.35	0.14	0.04	0.02
Range	0.00 - 1.21	0.00 - 0.82	0.00 - 0.60	0.00 - 0.91
% Reduction	---	60	88	95
P Value (M-W)	---	0.0153	< 0.0001	< 0.0001
N	29	33	29	30

Astrocytosis: The extent of astrocytosis in
the retrosplenial cortex was quantified as previously
described (55). The treatment groups containing GM-CSF
showed significantly reduced astrocytosis (Figure 6).

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Example 9
C4(E9V)-V3_{89.6P} Peptide Immunization
of Cynomologous Macaques

5   The objective of this experiment was to
evaluate the immunogenicity of an HIV-1 Env-derived
peptide conjugate, C4(E9V)-V3$_{89.6P}$, administered with and
without an adjuvant combination formulation of this
invention in another primate species, cynomologous
10   monkeys (*Macaca fascicularis*). The C4-V3$_{89.6P}$ peptide
described in Example 7 was modified by changing the
glutamic acid at amino acid residue 9 to valine. The
resulting peptide conjugate, designated C4(E9V)-V3$_{89.6P}$,
was used, and has the following sequence:
15   Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly
             Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn
             Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg
             Ala Phe Tyr Ala Arg Arg (SEQ ID NO:5)
The glutamic acid to valine substitution (E9V) within
20   the C4 region of the peptide increases the
immunogenicity of the peptide above that of the
unmodified sequence in the mouse model.
             This HIV-1 Env-derived peptide is capable of
eliciting humoral immune responses in mice. However,
25   due to the difficulty of extrapolating mouse results to
humans, it is necessary to test potential HIV-1
immunogenic compositions in non-human primates before
proceeding into Phase I human clinical trials. In this
experiment, both intramuscular (IM) and intranasal (IN)
30   routes of administration were evaluated. Animals were
immunized five times at weeks 0, 4, 8, 18 and 23. On a
weekly basis through week 25, blood samples and
cervicovaginal and mucosal washes were collected and
analyzed for the presence of antibodies to the
35   immunogenic composition.

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Experimental Design: A total of eight cynomologous monkeys, four animals per group (Table 28), was used for the experiment. Group 1 received no adjuvant; Group 2 received the adjuvant formulation 529 SE plus GM-CSF. Animals are being housed and evaluated at an animal care facility.

Table 28
529 SE plus GM-CSF vs. no adjuvant

Group #	Immunogen	Adjuvant	Route
1	C4(E9V)-V3$_{89.6P}$ peptide	none	IN
2	C4(E9V)-V3$_{89.6P}$ peptide	529SE/GM-CSF	IM

Immunizations: All intranasal immunizations were delivered with a 100 μl metered dose nasal spray device. All intramuscular injections were given in the quadriceps muscle with needle and syringe. All animals were immunized on a schedule of 0, 4, 8, 18 and 23 weeks.

Formulations:

Group 1: 1000 μg C4 (E9V)-V3$_{89.6P}$ peptide in sterile normal saline, final volume 200μl (100μl each nostril).

Group 2: 1000 μg C4 (E9V)-V3$_{89.6P}$ peptide, 50 μg 529 SE, and 250 μg human GM-CSF, final oil concentration 1%, final volume 1.0 ml (500μl each quadriceps by IM injection).

Assays to monitor immunogen-induced immune responses:

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Immediately before and after immunization, all animals were closely monitored for immunogen-induced humoral immune responses by the following assays:

5   (1) Serum anti-C4(E9V)-V3$_{89.6P}$ peptide IgG serum antibody titers by ELISA.
           (2) Mucosal (cervicovaginal, nasal) anti-C4(E9V)-V3$_{89.6P}$ peptide IgG antibody titers by ELISA.

10   <u>Results</u>:

<u>Immunogen Safety and Tolerability</u>:

           The C4(E9V)-V3$_{89.6P}$ peptide when administered
15 alone or in combination with the adjuvants 529 SE/GM-CSF was extremely well tolerated. For the animals immunized by the intramuscular route using needle and syringe, no adverse injection site reactivities were noted. All animals were closely monitored for changes in body
20 temperature during the 24 hours immediately following each administration of the immunogen. At no time during the study, did any of the animals demonstrate abnormally elevated body temperature readings (data not shown).

25 <u>Immunogen-specific Serum antibody responses</u>:

           Serum samples from all animals were obtained immediately prior to and one and two weeks after each immunization (through 25 weeks). Two weeks after the
30 final immunization, all the serum samples were analyzed for the presence of anti-C4(E9V)-V3 peptide IgG antibodies. The intranasally immunized group 1 animals (C4(E9V)-V3$_{89.6P}$ peptide alone) failed to demonstrate serum anti-C4(E9V)-V3$_{89.6P}$ IgG antibody titers higher
35 than pre-immune levels (Figure 7). In contrast, the group 2 animals (IM administration of C4(E9V)-V3$_{89.6P}$ +

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529 SE/GM-CSF) developed significant levels of serum C4(E9V)-V3-specific IgG antibodies (Figure 7). The reported geometric mean endpoint titer is calculated using the lowest titer from each individual animal that is 3-fold over the reading for pooled naïve serum at the same dilution.

Group 1 animals (without adjuvant) had anti-C4(E9V)-V3$_{89.6P}$ IgG antibody titers in cervicovaginal lavage samples that were higher than pre-immune levels only after the fourth immunization, but declined thereafter (Figure 8). In contrast, group 2 animals (with adjuvant) had anti- C4(E9V)-V3$_{89.6P}$ IgG antibody titers in cervicovaginal lavage samples that were higher than pre-immune levels after the first immunization and increased after each subsequent immunization (although there was some later drop-off in each case) (Figure 8).

Group 1 animals (without adjuvant) failed to demonstrate anti-C4(E9V)-V3$_{89.6P}$ IgG antibody titers in nasal washes higher than pre-immune levels (Figure 9). In contrast, group 2 animals (with adjuvant) developed significant levels of anti-C4(E9V)-V3$_{89.6P}$ IgG antibody titers in nasal wash samples (Figure 9).

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What is claimed is:

1.   An antigenic composition comprising a
selected antigen from a pathogenic virus, bacterium,
fungus or parasite, or from a cancer cell or tumor
cell, or from an allergen, or from a self molecule, and
an effective adjuvanting amount of the combination of:
(1) an aminoalkyl glucosamine phosphate compound (AGP),
or a derivative or analog thereof, and (2) a cytokine
or lymphokine, or an agonist to said cytokine or
lymphokine, wherein the combination of adjuvants
enhances the immune response in a vertebrate host to
said antigen.

2.   The antigenic composition of Claim 1
where the selected antigen is a polypeptide, peptide or
fragment derived from a protein.

3.   The antigenic composition of Claim 1
where the AGP is used in the form of a stable oil-in-
water emulsion.

4.   The antigenic composition of Claim 1
where the cytokine or lymphokine is selected from the
group consisting of granulocyte macrophage colony
stimulating factor and interleukin-12.

5.   The antigenic composition of Claim 4
where the cytokine or lymphokine is granulocyte
macrophage colony stimulating factor.

6.   The antigenic composition of Claim 5
where the AGP is used in the form of a stable oil-in-
water emulsion.

7.   The antigenic composition of Claim 4
where the cytokine or lymphokine is interleukin-12.

8.   The antigenic composition of Claim 7
where the AGP is used in the form of a stable oil-in-
water emulsion.

9.   The antigenic composition of Claim 1
which further comprises a diluent or carrier.

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      10.   The antigenic composition of Claim 9 where the AGP is used in the form of a stable oil-in-water emulsion.

      11.   The antigenic composition of Claim 1 where the AGP is:
2-[(R)-3-Tetradecanoyloxytetradecanoylamino]ethyl 2-Deoxy-4-O-phosphono-3-O-[(R)-3-tetradecanoyoxytetradecanoyl]-2-[(R)-3-tetradecanoyoxytetradecanoylamino]-β-D-glucopyranoside (529).

      12.   The antigenic composition of Claim 1 where the selected antigen is from human immunodeficiency virus (HIV).

      13.   The antigenic composition of Claim 12 where the selected HIV antigen is an HIV protein, polypeptide, peptide or fragment derived from said protein.

      14.   The antigenic composition of Claim 13 where the selected antigens are HIV peptides selected from the group consisting of peptides having the amino acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID NO:1);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID NO:2);
Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu (SEQ ID NO:3);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID NO:4); and

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Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:5).
       15.   The antigenic composition of Claim 14
wherein the HIV peptide is the peptide having the amino
acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile
His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:1).
       16.   The antigenic composition of Claim 14
wherein the HIV peptide is the peptide having the amino
acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:2).
       17.   The antigenic composition of Claim 14
wherein the HIV peptide is the peptide having the amino
acid sequence:
Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val
Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu
(SEQ ID NO:3).
       18.   The antigenic composition of Claim 14
wherein the HIV peptide is the peptide having the amino
acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:4).
       19.   The antigenic composition of Claim 14
wherein the HIV peptide is the peptide having the amino
acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser

Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID NO:5).

     20.   The antigenic composition of Claim 12 where the AGP is used in the form of a stable oil-in-water emulsion.

     21.   The antigenic composition of Claim 12 where the cytokine or lymphokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and interleukin-12.

     22.   The antigenic composition of Claim 21 where the cytokine or lymphokine is granulocyte macrophage colony stimulating factor.

     23.   The antigenic composition of Claim 22 where the AGP is used in the form of a stable oil-in-water emulsion.

     24.   The antigenic composition of Claim 21 where the cytokine or lymphokine is interleukin-12.

     25.   The antigenic composition of Claim 24 where the AGP is used in the form of a stable oil-in-water emulsion.

     26.   The antigenic composition of Claim 12 which further comprises a diluent or carrier.

     27.   The antigenic composition of Claim 26 where the AGP is used in the form of a stable oil-in-water emulsion.

     28.   The antigenic composition of Claim 12 where the AGP is 529.

     29.   A method for increasing the ability of an antigenic composition containing a selected antigen from a pathogenic virus, bacterium, fungus or parasite to elicit the immune response of a vertebrate host, which comprises administering to said host an antigenic composition of Claim 1.

     30.   A method for increasing the ability of an antigenic composition containing a selected antigen from a pathogenic virus, bacterium, fungus or parasite

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to elicit the immune response of a vertebrate host,
which comprises administering to said host an antigenic
composition of Claim 9.

31. A method for increasing the ability of
an antigenic composition containing an HIV antigen to
elicit the immune response of a vertebrate host, which
comprises administering to said host an antigenic
composition of Claim 12.

32. A method for increasing the ability of
an antigenic composition containing an HIV antigen to
elicit the immune response of a vertebrate host, which
comprises administering to said host an antigenic
composition of Claim 26.

33. The method of Claim 32 where the
selected antigens are HIV peptides selected from the
group consisting of peptides having the amino acid
sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile
His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:1);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:2);
Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val
Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu
(SEQ ID NO:3);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:4); and
Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:5).

34. The method of Claim 33 wherein the HIV peptide is the peptide having the amino acid sequence: Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID NO:1).

35. The method of Claim 33 wherein the HIV peptide is the peptide having the amino acid sequence: Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID NO:2).

36. The method of Claim 33 wherein the HIV peptide is the peptide having the amino acid sequence: Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu (SEQ ID NO:3).

37. The method of Claim 33 wherein the HIV peptide is the peptide having the amino acid sequence: Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID NO:4).

38. The method of Claim 33 wherein the HIV peptide is the peptide having the amino acid sequence: Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID NO:5).

39. A method for increasing the ability of an antigenic composition containing a selected antigen from a pathogenic virus, bacterium, fungus or parasite to elicit cytotoxic T lymphocytes in a vertebrate host, which comprises administering to said host an antigenic composition of Claim 1.

40.   A method for increasing the ability of
an antigenic composition containing a selected antigen
from a pathogenic virus, bacterium, fungus or parasite
to elicit cytotoxic T lymphocytes in a vertebrate host,
which comprises administering to said host an antigenic
composition of Claim 9.

41.   A method for increasing the ability of
an antigenic composition containing an HIV antigen to
elicit cytotoxic T lymphocytes in a vertebrate host,
which comprises administering to said host an antigenic
composition of Claim 12.

42.   A method for increasing the ability of
an antigenic composition containing an HIV antigen to
elicit cytotoxic T lymphocytes in a vertebrate host,
which comprises administering to said host an antigenic
composition of Claim 26.

43.   The method of Claim 42 where the
selected antigens are HIV peptides selected from the
group consisting of peptides having the amino acid
sequence:

Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile
His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:1);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:2);
Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val
Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu
(SEQ ID NO:3);
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:4); and

Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:5).

    44.   The method of Claim 43 wherein the HIV
peptide is the peptide having the amino acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Cys Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile
His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:1).

    45.   The method of Claim 43 wherein the HIV
peptide is the peptide having the amino acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Tyr Asn Lys Arg Lys Arg Ile His
Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr Lys (SEQ ID
NO:2).

    46.   The method of Claim 43 wherein the HIV
peptide is the peptide having the amino acid sequence:
Arg Gln Ile Ile Asn Thr Trp His Lys Val Gly Lys Asn Val
Tyr Leu Glu Gly Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu
(SEQ ID NO:3).

    47.   The method of Claim 43 wherein the HIV
peptide is the peptide having the amino acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:4).

    48.   The method of Claim 43 wherein the HIV
peptide is the peptide having the amino acid sequence:
Lys Gln Ile Ile Asn Met Trp Gln Val Val Gly Lys Ala Met
Tyr Ala Thr Arg Pro Asn Asn Asn Thr Arg Glu Arg Leu Ser
Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg Arg (SEQ ID
NO:5).

    49.   A method for increasing the ability of
an antigenic composition containing a selected cancer
antigen or tumor-associated antigen from a cancer cell

or tumor cell to elicit a therapeutic or prophylactic anti-cancer effect in a vertebrate host, which comprises administering to said host an antigenic composition comprising said selected cancer antigen or tumor-associated antigen from a cancer cell or tumor cell, and an effective adjuvanting amount of the combination of: (1) an AGP, or a derivative or analog thereof, and (2) a cytokine or lymphokine, or an agonist to said cytokine or lymphokine.

50. A method for increasing the ability of an antigenic composition containing a selected allergen to moderate an allergic response in a vertebrate host, which comprises administering to said host an antigenic composition comprising said allergen, and an effective adjuvanting amount of the combination of: (1) an AGP, or a derivative or analog thereof, and (2) a cytokine or lymphokine, or an agonist to said cytokine or lymphokine.

51. An antigenic composition containing a selected antigen from a molecule or portion thereof which represents those produced by a host in an undesired manner, amount or location so as to reduce such an undesired effect, by including an effective adjuvanting amount of the combination of: (1) an AGP, or a derivative or analog thereof, and (2) a cytokine or lymphokine, or an agonist to said cytokine or lymphokine.

52. The antigenic composition of Claim 51, where the selected antigen is a polypeptide, peptide or fragment derived from amyloid precursor protein, or an antibody thereto.

53. The antigenic composition of Claim 52, where the selected antigen is the Aβ peptide, which is an internal, 39-43 amino acid fragment of amyloid precursor protein, or a fragment of the Aβ peptide.

54. The antigenic composition of Claim 53, where the selected antigen is the Aβ peptide having the amino acid sequence:
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala (SEQ ID NO:6).

55. The antigenic composition of Claim 54, where the AGP is 529.

56. An method for increasing the ability of an antigenic composition containing a selected antigen from a molecule or portion thereof which represents those produced by a host in an undesired manner, amount or location so as to reduce such an undesired effect, by including an effective adjuvanting amount of the combination of: (1) an AGP, or a derivative or analog thereof, and (2) a cytokine or lymphokine, or an agonist to said cytokine or lymphokine.

57. A method for increasing the ability of an antigenic composition to prevent or treat disease characterized by amyloid deposition in a vertebrate host, which comprises administering to said host a polypeptide, peptide or fragment derived from amyloid precursor protein, or an antibody thereto, and an effective adjuvanting amount of the combination of: (1) an AGP, or a derivative or analog thereof, and (2) a cytokine or lymphokine, or an agonist to said cytokine or lymphokine.

58. The method of Claim 57 where the selected antigen is the Aβ peptide, which is an internal, 39-43 amino acid fragment of amyloid precursor protein, or a fragment of the Aβ peptide.

59. The method of Claim 58 where the selected antigen is the Aβ peptide having the amino acid sequence:
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His

Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala
(SEQ ID NO:6).

      60.   The method of Claim 59 where the AGP is
529.

      61.   An adjuvant formulation comprising
the combination of: (1) an aminoalkyl glucosamine
phosphate compound (AGP), or a derivative or analog
thereof, and (2) a cytokine or lymphokine, or an
agonist to said cytokine or lymphokine.

      62.   The adjuvant formulation of Claim 61
where the AGP is used in the form of a stable oil-in-
water emulsion.

      63.   The adjuvant formulation of Claim 61
where the cytokine or lymphokine is selected from the
group consisting of granulocyte macrophage colony
stimulating factor and interleukin-12.

      64.   The adjuvant formulation of Claim 61
which further comprises a diluent or carrier.

      65.   The adjuvant formulation of Claim 61
where the cytokine or lymphokine is granulocyte
macrophage colony stimulating factor.

      66.   The adjuvant formulation of Claim 61
where the cytokine or lymphokine is interleukin-12.

      67.   The adjuvant formulation of Claim 61
where the AGP is 529.

FIGURE 1

Comparison of GMT's of GM-CSF
Formulations to MPL-SE Alone

FIGURE 2

Cortical Total Abeta Levels

FIGURE 3

FIGURE 4

Frontal Cortex Amyloid Burden

FIGURE　5

Frontal Cortex Neuritic Burden

FIGURE 6

Retrosplenial Cortical Astrocytosis Median Values

PBS	MPL-SE	MPL-SE/ gmcsf	RC529/ gmcsf
2.2049	2.0839	1.6070	1.3659
p-value	0.3703	0.0131	0.006

FIGURE 7

C4(E9V)-V3 Peptide-specific IgG Ab Titer in Serum
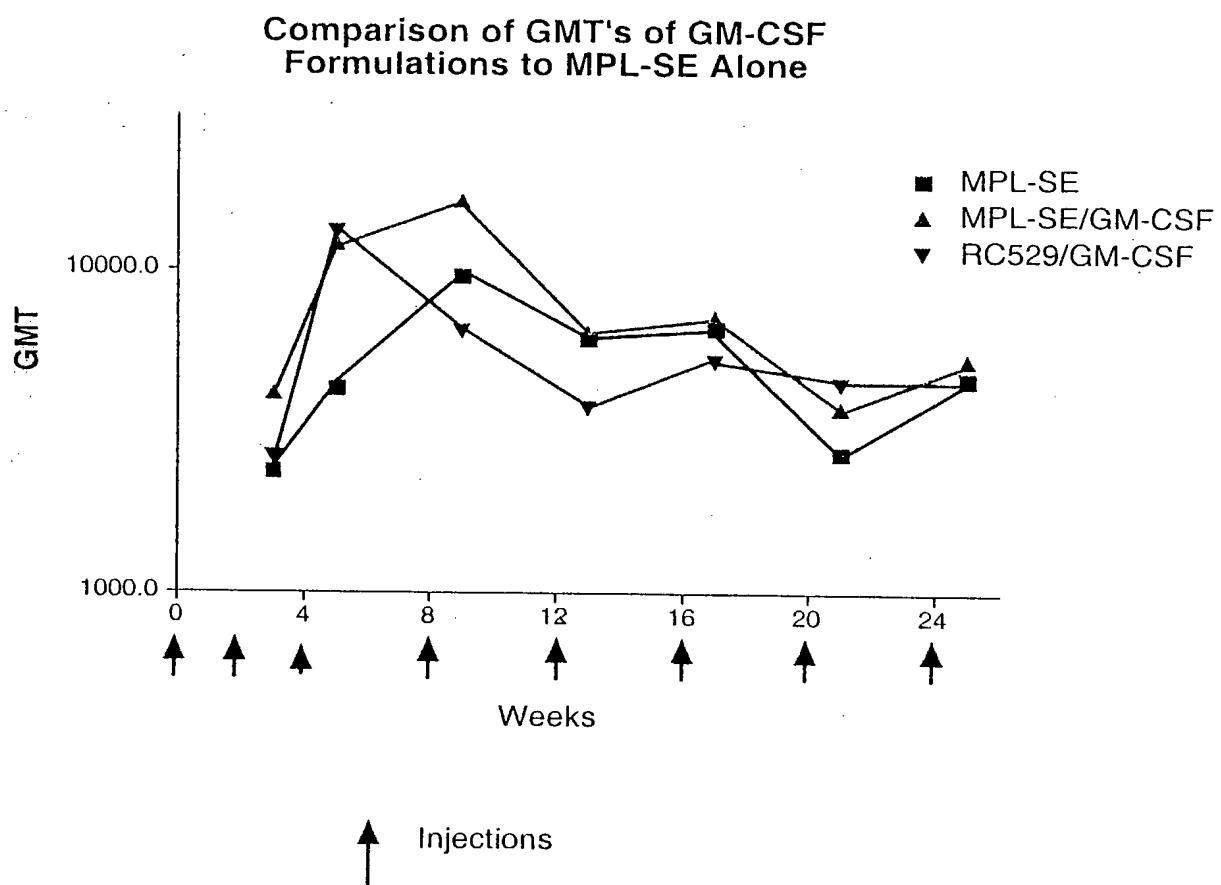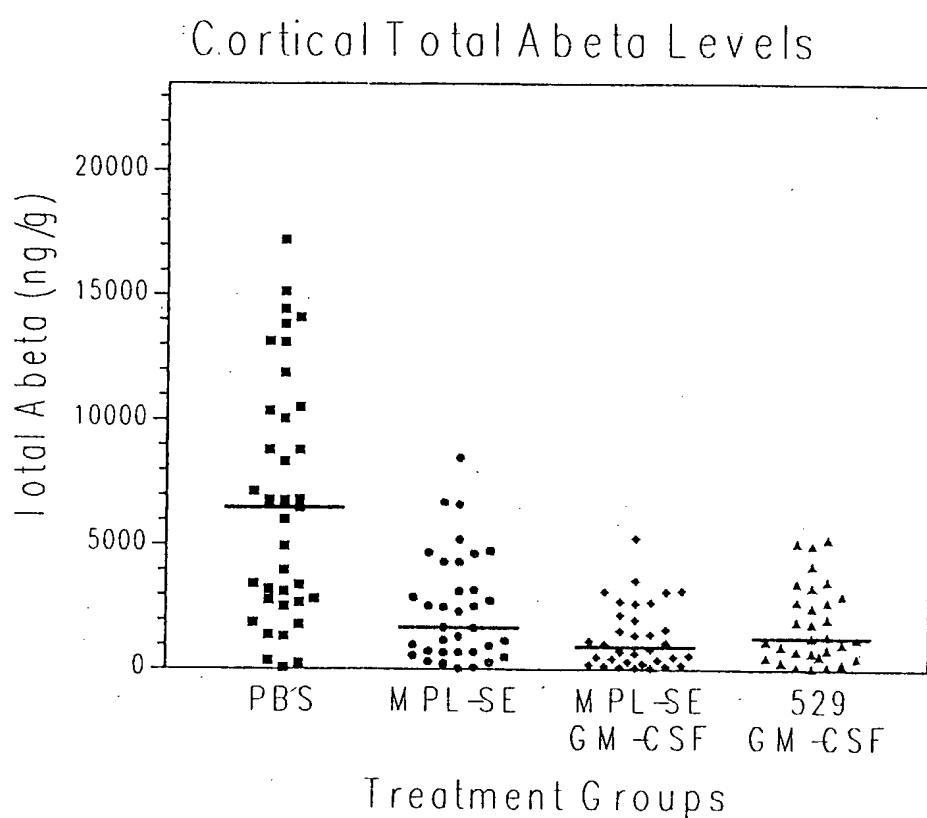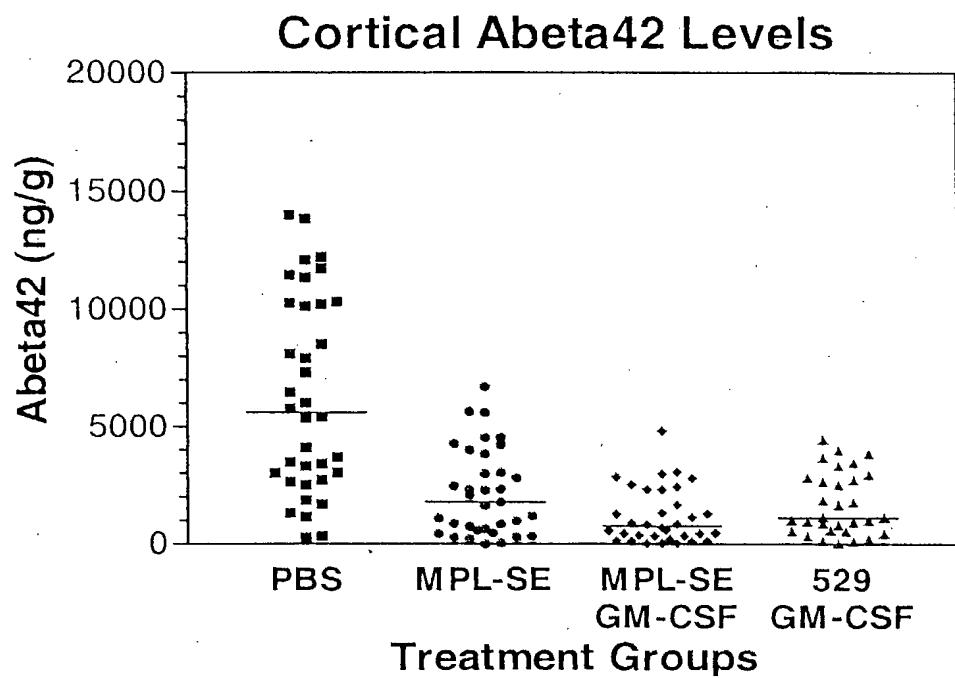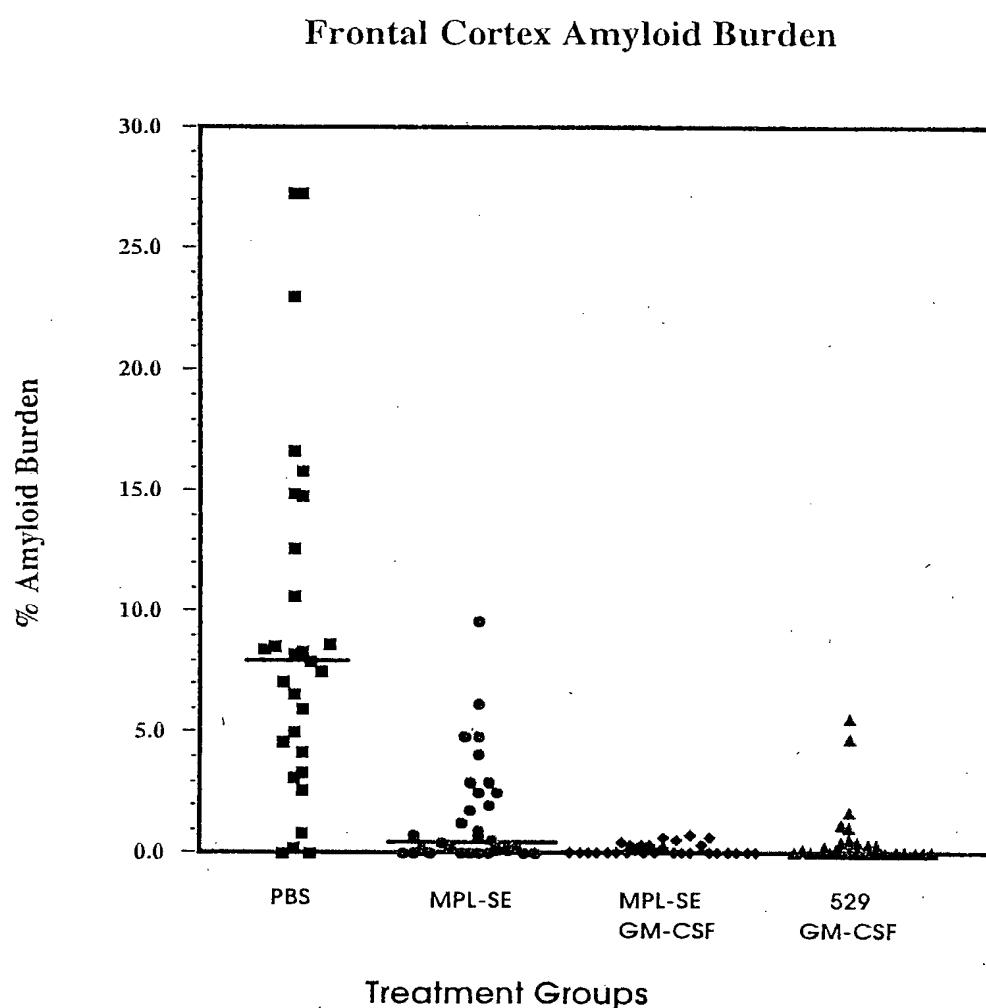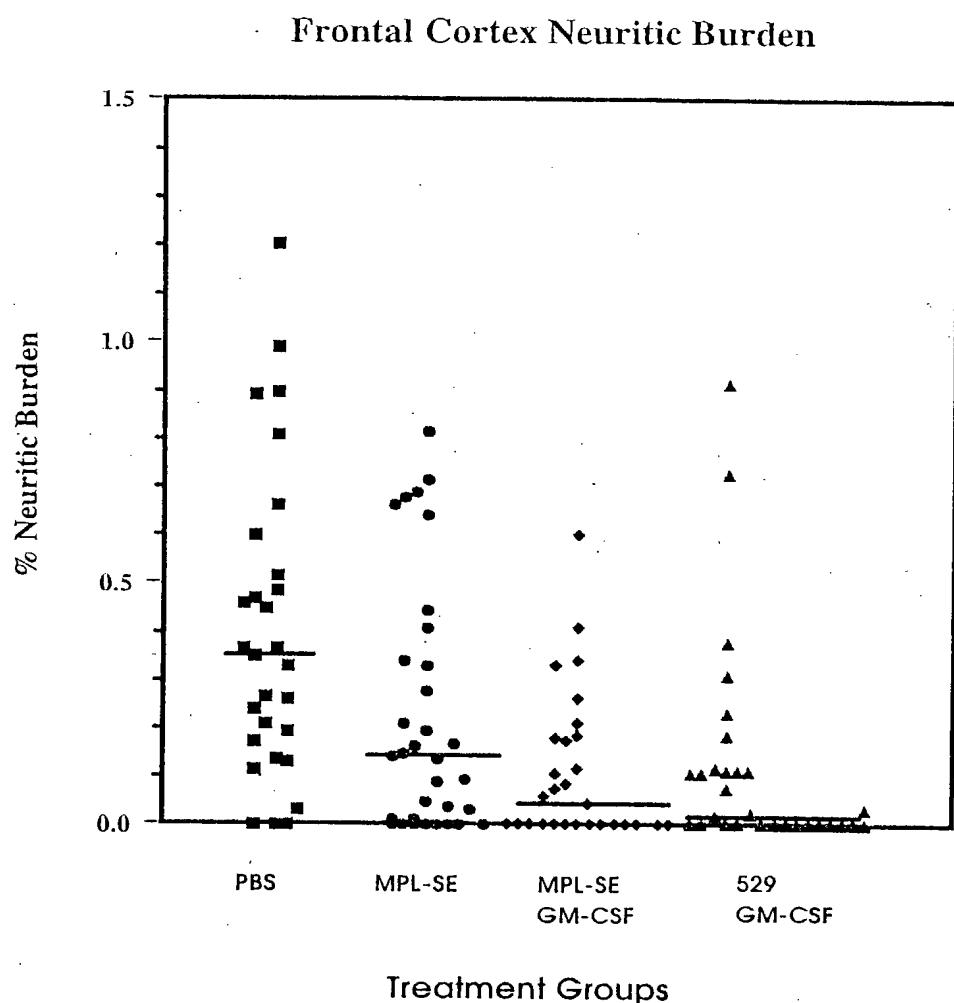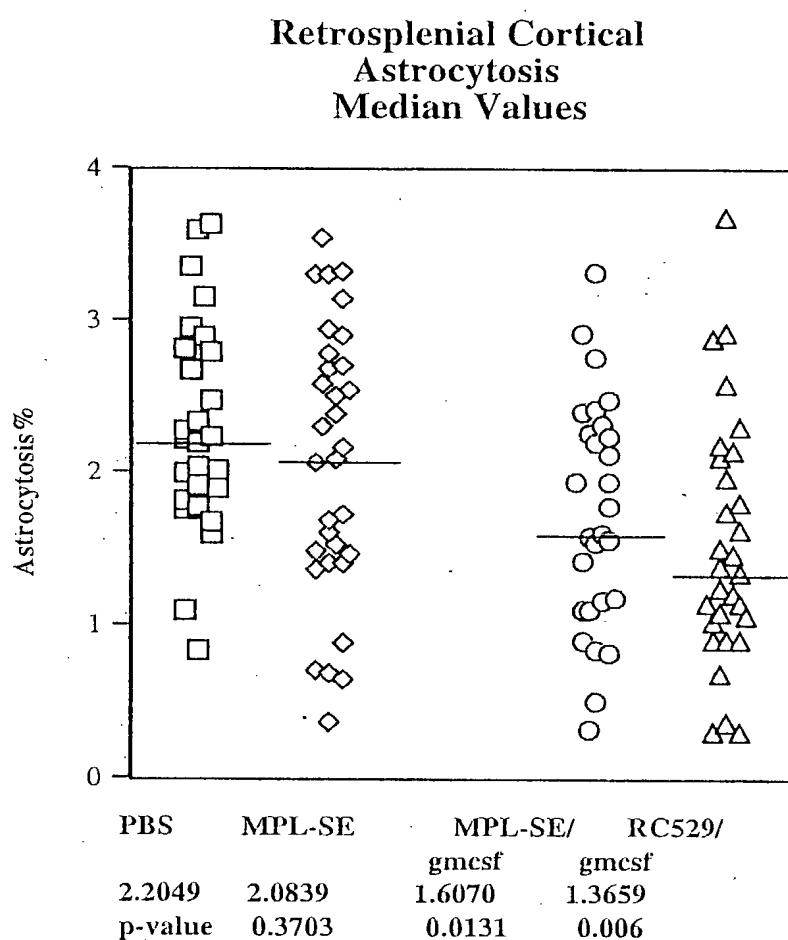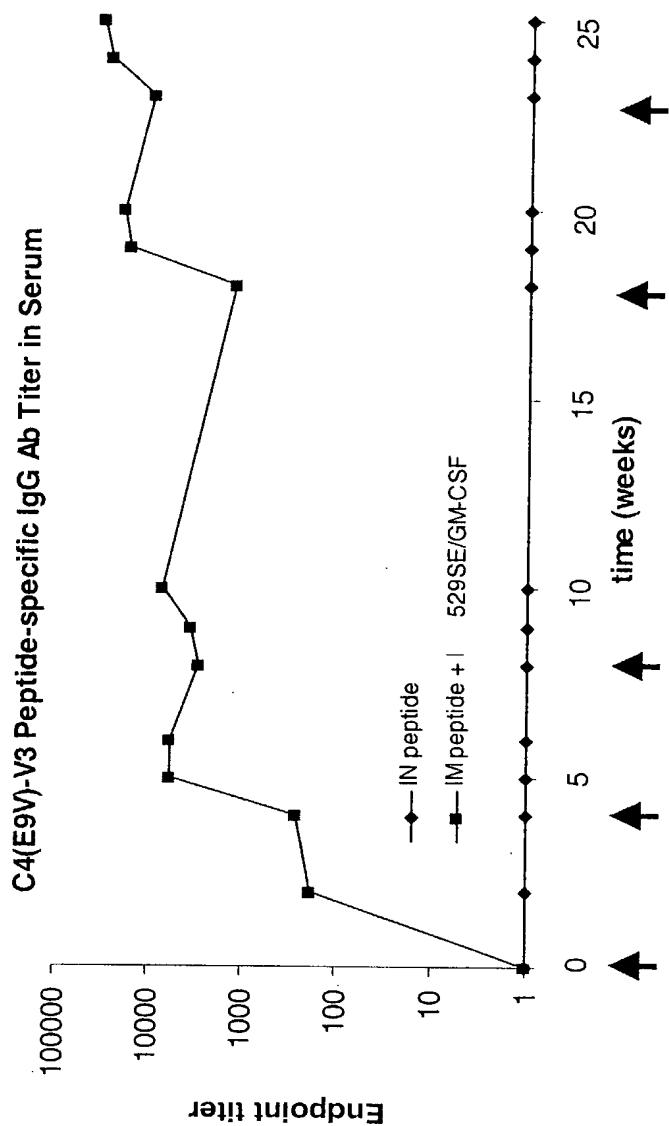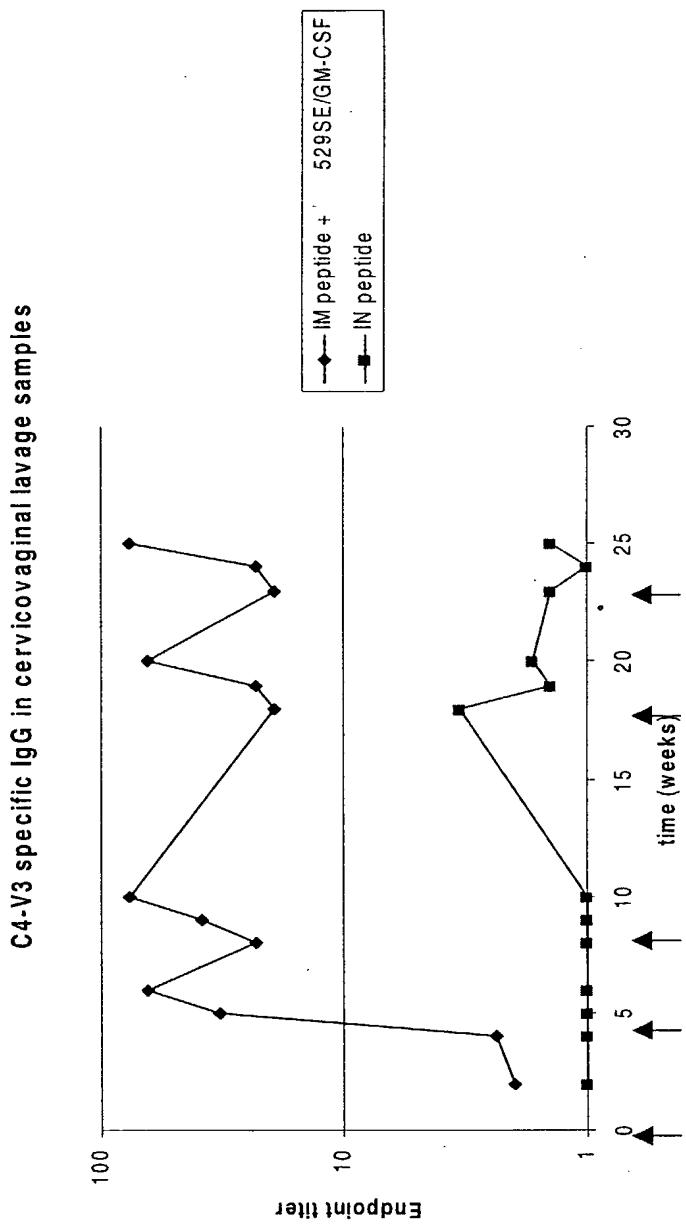
FIGURE 8

FIGURE 9

C4-V3 specific IgG in nasal wash samples
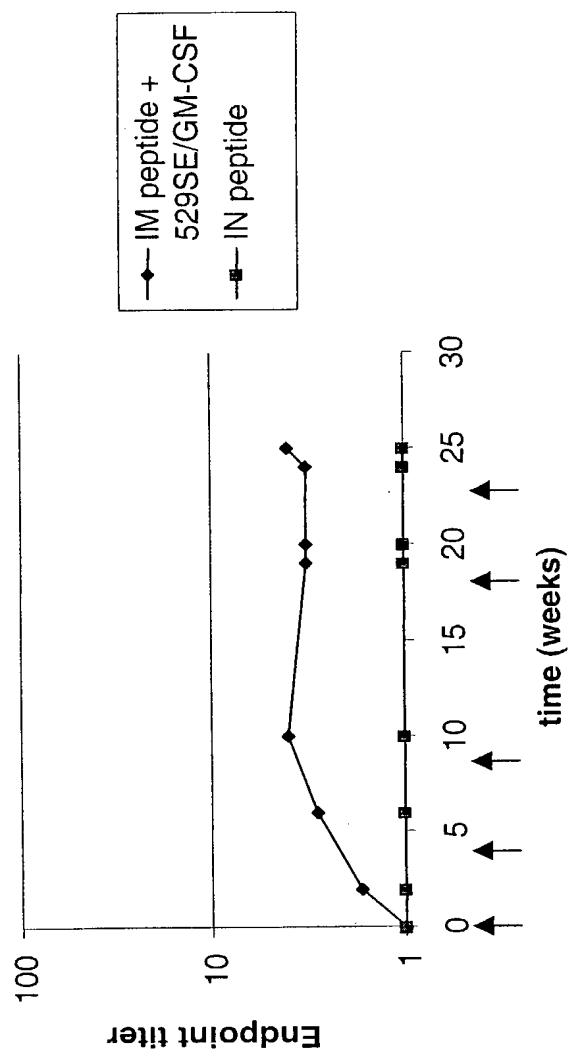
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            20   25   30

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            20                  25                  30


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Gly Leu Met Val Gly Gly Val Val Ile Ala
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